PHYSIOLOGICAL ECOLOGY OF BIOCRUST MOSSES UNDER GLOBAL CHANGE: IMPLICATIONS FOR DRYLAND BIOGEOCHEMISTRY

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Kirsten Kryder Coe

August 2012

PHYSIOLOGICAL ECOLOGY OF BIOCRUST MOSSES UNDER GLOBAL CHANGE: IMPLICATIONS FOR DRYLAND BIOGEOCHEMISTRY

Kirsten Kryder Coe, Ph. D.

Cornell University 2012

Increased atmospheric CO_2 is changing the energy balance of the Earth and altering global precipitation. Drylands cover >40% of the terrestrial surface and may be among the most responsive ecosystems to these changes. My dissertation addresses the influences of global change on biocrust mosses, a group of plants instrumental to biogeochemistry in drylands.

First, I provide an overview of the physiological ecology of biocrust mosses. These plants are adapted to pulse-dynamic water and resource availability in drylands because they are desiccation tolerant, which (1) enables carbon fixation during precipitation events, and dormancy (including protection from light and temperature extremes) between them, but (2) places constraints on performance owing to the costs of responding to precipitation, resulting in vulnerability to changes in precipitation regime.

Second, I examine the physiological effects of 10 years elevated CO_2 and high temperature events on biocrust mosses. I show that, unlike vascular plants, mosses do not acclimate photosynthetically to elevated CO_2. High temperatures reduce photosynthetic performance, but elevated CO_2 exposure partially alleviates this stress, potentially reflecting reallocation of nitrogen to cellular components that offer photosynthetic thermotolerance.

Third, I study the influence of changes in precipitation event size, frequency, and seasonality on moss carbon balances from precipitation events. Precipitation event size was

positively correlated with (and was the strongest driver of) carbon balance because mosses remain photosynthetically active longer during large events. Rainfall frequency was negatively correlated with carbon balance because the cost to regain functional capacity during a precipitation event is higher after longer dry periods. Finally, carbon balance varied over the course of the year, even for events of identical magnitude, indicating that intrinsic physiological differences are present across seasons than influence response to precipitation.

Lastly, I combine physiology with meteorological records from Western North America to create a predictive model for biocrust moss functioning in the next 100 years. Using simulations, I show that subtle shifts in intra-annual precipitation can drive long-term performance, rare large precipitation events can change the trajectory of survival, and most projected dryland scenarios will result in reduced performance in biocrust mosses.

BIOGRAPHICAL SKETCH

Kirsten Kryder Coe was born in October 1982 in Baltimore, Maryland, where she grew up in Windsor Hills, a neighborhood that borders one of the largest urban parklands in the United States, Gwynns Falls Leakin Park, and harbors a diversity of socially and environmentally conscious citizens.

Kirsten was introduced to the natural world at an early age, and spent much of her youth hiking in the Appalachian Mountains, and traveling with her family to National Parks in the Southwestern United States. Kirsten attended a Quaker school in Baltimore from kindergarten through grade 12, during which time the important values of simplicity, patience, and cooperation were (albeit not too subtly) instilled, and still carry great importance in her life today. As a high school senior, Kirsten elected to double-up on her science requirement and signed up for an Ecology class, during which she discovered she had a penchant for plant taxonomy and field identification. Although plant ecology came extremely naturally to Kirsten, it wasn't until several years later that it developed into her career path.

In 2000, Kirsten began college at Syracuse University, rapidly majored in Geology, began work in the Syracuse University Stable Isotope Lab, and even won an undergraduate Earth Science academic excellence award as a freshman, before transferring to SUNY College of Environmental Science and Forestry (ESF) for the remainder of her degree. ESF became a perfect academic home for Kirsten because it was comprised of students and professors that deeply cared about biology, education, and environmental stewardship. Two classes in particular shaped both Kirsten's time at ESF, and her future as an academic. In The Ecology of Mosses, Kirsten was introduced to an extraordinary, miniature world of plants from which myriad

ecological questions seemed to arise, many of which were still unanswered! In The Deserts of Southern Africa, Kirsten traveled to South Africa and Namibia where she learned about the diverse adaptations of dryland organisms to withstand harsh environmental conditions, and completed an independent study on the leaf physiology of the ancient desert gymnosperm *Welwitschia mirabilis* in the Namib Desert. Her experiences during this course led her to take a summer research position at the University of Kwazulu Natal in South Africa, where she worked in the field on projects examining stress physiology in bush babies and elephant shrews. Before graduating *summa cum laude* from ESA in December 2003, Kirsten, unable to stray too far from bryophytes, completed another independent study on habitat partitioning of calciphilic mosses on limestone outcrops in central New York.

Before beginning her graduate program at Cornell University, Kirsten first spent a summer working for the National Park Service near Anchorage, AK, and also worked as a field assistant on a University of Alaska project examining the impacts of nitrogen deposition on feathermoss productivity in the boreal forest understory. Kirsten then stayed in Anchorage for an additional year during which time she was employed in commercial construction, working long hours and developing useful engineering skills that would later come to be invaluable in the design of specialized field equipment for ecophysiology research. Then, in 2005, Kirsten worked in Botswana as the manager of a safari lodge in the Okavango Delta. The remote lodge was located inside one of the largest intact wilderness areas in Africa and was only accessible by small plane. Because the lodge was unfenced, it was not uncommon to run into elephant, baboon, and giraffe on the grounds by day, and find the tracks of lion, leopard, and hippo that had walked through the camp by night. The experiences and life skills Kirsten gained while living immersed

in another culture in Botswana (and being responsible for the health, welfare and safety of the on-site camp staff as well as tourists) were extremely rewarding and unforgettable.

Kirsten began her PhD research with Dr. Jed Sparks at Cornell in fall 2006. During her time as a graduate student, Kirsten learned an immense amount about designing and conducting ecological research, but learned an equal amount about herself as a person, teacher, and academic. Kirsten's PhD thesis topic, the influence of global change on the physiological ecology of dryland biocrust mosses, seemed to develop seamlessly and logically from all of her meaningful past research and coursework experiences. She relished the opportunity to conduct research in the gorgeous, vast, and grand Southwestern U.S., working with colleagues at the USGS "crust lab" in Moab, UT. Over the years at Cornell, Kirsten's research questions expanded in scope, and through developing an understanding of the physiological mechanisms underling response to global change as well as the ecosystem-scale consequences of such changes, she developed a truly holistic understanding of global change in dryland systems.

One of the most rewarding aspects of Kirsten's time as a PhD student was the vast array of opportunities to gain teaching experience and expertise. Kirsten was a TA for eight different biology courses, and was awarded the CALS Teaching Award twice. Kirsten also spent two summers teaching Ecology to prisoners Auburn Maximum Security Prison with the Cornell Prison Education Program (CPEP). Kirsten designed and taught the first general Ecology course offered by CPEP, and also worked to create the first ever educational garden on prison grounds to be used by incarcerated students for field experimentation. She will soon publish an article based on her experiences at Auburn titled "Ecology behind bars: A teaching garden cultivates free minds" in the journal *Radical Teacher*.

Upon finishing her PhD, Kirsten will work as a postdoctoral associate with Dr. Ted Schuur, at the University of Florida, where she will be examining the consequences of permafrost thaw in the Alaskan arctic on terrestrial carbon cycling and feedbacks to global change. On the longer term, she looks forward to thoughtfully building a fulfilling career as an ecophysiologist and ecosystem scientist, and starting a family with her partner, Petra.

DEDICATION

I dedicate this dissertation to my mother, Julee Hemington Kryder-Coe

ACKNOWLEDGMENTS

This work would not have been possible without the generous support of family, friends, colleagues, and mentors.

First and foremost, Jed Sparks has been a fantastic advisor throughout the duration of my time at Cornell. Jed has been committed to my success as a researcher, student, teacher, and academic from the very beginning. I am thankful for the freedom he always gave me to pursue my research goals independently, the helpful reminders of the broader context of my work, and the assistance in articulating my work in written as well as presentation formats. I also very much appreciate Jed as a person; he has a good heart and it showed in his interactions with me.

The entire Sparks lab over the years has been a great community in which to learn and grow as a scientist. It has been very stimulating and challenging to be a part of a group that has shared interests and also takes on a broad array of research topics. The interactions I have had with my lab group have helped make me a confident plant physiological ecologist and biogeochemist.

My other Ph.D. committee members were also incredibly helpful throughout this process. Barbara Bedford always helped me see the place my work fit within the overlapping scientific communities of biogeochemistry, plant ecology, and ecophysiology. Barbara was also one of my most important mentors at Cornell, and I am grateful for her advice during the difficult parts of this journey. Tom Whitlow has encouraged me to think outside the box in my research, and has shown me how scientific tools, methodologies, and theories can be useful across disciplines.

There have been other Cornell professors that served as mentors throughout this process as well. Harry Greene has always opened his office door to me for conversations about my

research, teaching, folk music, or his travel and hunting adventures, and I have appreciated these moments very much. Harry has also been one of the most encouraging people for me through all of ups and downs of graduate school, and I think of him as a true friend. Steve Ellner was an instrumental part of the execution of the beginning stages of the modeling work presented in Chapter 4. I also have to thank Rob Raguso and Anurag Agrawal for keeping me well rounded as an ecologist with many stimulating conversations on a variety of topics over the years.

Lloyd Stark (University of Nevada Las Vegas) has been a fantastic colleague, friend, and fellow bryologist nerd over the years. His knowledge of dryland moss ecology is unmatched, and he has been an exceptional resource for me in my research.

The USGS Canyonlands Research Station (the "Crust Lab") in Moab, UT was the site where the majority of my Ph.D. research was conducted. Jayne Belnap, the head P.I. for the facility throughout my time there, has been an amazing collaborator on my work. She introduced me to my research topic by showing me the effects of changing precipitation on biocrust mosses, and the dramatic effects of moss removal on biocrust structure. Jayne, in addition to Jed, has also continually provided the important perspective of a true desert-dweller on my work. My research would absolutely not have been possible without the incredible engineering skills of Ed Grote, who designed the gas exchange system I used at the Crust Lab to measure CO_2 exchange from mosses. I am grateful for his expertise, and his patient instruction as I learned the ropes. Sasha Reed, also at the Crust Lab, has been there for countless insightful conversations about plant-soil interactions, and it has been a true joy working with her in recent years coupling my plant ecophysiology work to soil biogeochemistry. Finally, the numerous research technicians at the lab, especially Hilda Smith and Adam Kind, truly made this work possible by being trustworthy assistants during the prep phases of my experiments.

As an undergraduate at SUNY College of Environmental Science and Forestry, I was heavily influenced as an academic by two professors. First, Robin Kimmerer introduced me to the awe-inspiring field of bryology, and I am indebted to her for encouraging me to pursue my own research to fill in the current gaps of knowledge within that scientific field. Second, Scott Turner introduced me to dryland systems through a field course in southern Africa. I am thankful to still be in touch with him and feel his continued support as my academic journey moves forward.

One of the most rewarding and valuable aspects of my time as a graduate student at Cornell was the opportunity to teach biology to undergraduates. I am so thankful to all of the students in the eight different courses in which I served as a teaching assistant. Collectively, they helped me become a better and better teacher over the years. In addition to teaching Cornell undergraduates, I also spent two summers teaching an Ecology course that I developed to inmates at Auburn Maximum Security Prison through the Cornell Prison Education Program. It is impossible to overestimate the importance of this experience for me as a socially conscious academic. The men I taught at Auburn were some of the most inspiring and respectful students I have ever had, and the challenges of working in a carceral institution forced me to be an adaptive and creative teacher. These collective experiences have shaped me into the instructor I am today, and have helped form my vision of myself as an academic in a research institution.

My friendships, particularly with other Cornell graduate students, have been a wonderful part of my time as a graduate student. Without these relationships, my Ph.D. experience would have been far less colorful, fun, amusing, and meaningful. Also, I am grateful to have learned so much about their own research topics over our years of friendship! My officemates, Sara DeLeon

and Marjorie Weber, have been particularly fantastic friends and sounding boards for ideas and venting sessions alike on a +/- daily basis.

In my early years, my mother (Julee), father (John), and brother (Justin) and I spent much of our free time as a family out of doors. My father was a passionate naturalist, adept mountaineer, and great teacher about the natural world, and I have fond memories of riding on his shoulders through the woods as a child. My mother thoughtfully planned our numerous trips to national parks in the Western US, the English windswept countryside, and the island of St. John, all of which exposed all of my senses to new environments and enhanced my understanding of natural systems. I think my mother truly believed I could do *anything* I wanted to do, and her encouragement for my academic and professional goals matched that sentiment. Both of my parents were also politically active, informed citizens who worked for social equality throughout their lives, and have taught and inspired me to do the same. Although I lost them too early in life, their life's passions still inspire me to learn enthusiastically, teach thoughtfully, and be environmentally and socially conscious as I move forward in my career as an academic.

Petra, my incredibly loving partner and best friend, has been the one thing I could truly count on every single day. Her sincere supportiveness of my research and teaching has been an instrumental part of my successes in both of these areas, and I am happy to be forever in her dept for those gifts. She is also one of the most inspiring people, naturalists, thinkers, and scientists I have ever known, and she has helped me become a more informed and thoughtful ecologist as well as a better person overall. I am so grateful to be able to spend my life with such a brilliant and beautiful soul.

My research was financially supported by the USGS SCEP fellowship, the NSF IGERT – funded Cornell Biogeochemistry and Environmental Biocomplexity Small Grant program, the

Kieckheffer Adirondack Fellowship, and Cornell Graduate School Research Travel Grants. I also received support from the American Bryological and Lichenological Society (ABLS) and the Botanical Society of America (BSA) for my research.

TABLE OF CONTENTS

CHAPTER 1

Physiological Ecology of Dryland Biocrust Mosses[1]

[1] Originally published as **Coe, K.K.**, Sparks, J. & Belnap, J. (2012) Ecophysiology of soil crust mosses in dryland systems. In *Advances in Photosynthesis and Respiration: Photosynthesis of Bryophytes and Early Land Plants*. David Hanson & Steve Rice, Eds. Govindjee & Sharkey, Series Eds. Springer, Dordrecht.

Abstract

Soil biocrusts are assemblages of cyanobacteria, lichens, and mosses ubiquitous to arid and semi-arid (dryland) systems that offer an array of ecosystem services. Soil crust mosses are taxonomically diverse, account for up to 30% of crust cover, and offer large contributions to crust biogeochemical functionality, yet remain the least understood component of the community. Because of selective pressures of their growth environment, such species are highly desiccation tolerant, with the ability to withstand the loss of most cellular water for extended periods of time, during which metabolism is suspended. Biocrust mosses can also tolerate larger ranges of temperature, light, and cellular water content than mesic species, yet still remain sensitive to certain aspects of environmental alteration. For one, changes in precipitation regime are likely to heavily influence survival in dryland mosses. Rainfall, occurring as discrete periods of hydration in dryland systems, causes mosses to undergo wet-dry cycles that result in either a positive or a negative carbon balance. Carbon balance is a measure of performance during individual rainfall events, and is a metric for long-term viability. Recent work suggests rainfall event magnitude plays a large role in carbon balance, as does the frequency and seasonality with which events fall. Biocrust mosses are stimulated by elevated CO_2, yet may not acclimate photosynthetically to long-term enrichment. Interestingly, elevated CO_2 may favor stress tolerance at the expense of growth in biocrust moss, particularly at high temperatures. Finally, despite low annual growth rates, nitrogen appears to place physiological limitations on reproductive biology of biocrust mosses. High levels of nitrogen deposition, however, have been shown to cause toxicity, competitive exclusion by vascular plants, and can reduce cyanosymbioses.

Introduction

Soil biocrusts, also known as cryptobiotic crusts or microbiotic crusts, are assemblages of organisms living amongst soil particles within the top few centimeters of soil and at the soil surface. Crusts are composed primarily of cyanobacteria, lichen, and moss (Rosentreter *et al.* 2007), and these components exist in various proportions depending on microclimate and disturbance regime of the soil environment. In dryland ecosystems, crusts increase the water holding capacity of soils, reduce erosion (Belnap 2003; 2006), and influence seedling establishment of grasses and shrubs (Johansen 1993). Biocrusts also influence elemental cycling in drylands (Housman et al. 2006), as they (a) contain a large proportion of photosynthesizing organisms and supply organic carbon to underlying soil; (b) contain free-living and lichenized cyanobacteria that fix atmospheric nitrogen (N_2) into a biologically available form (NH_4^+) (Evans and Belnap 1999); and (c) secrete compounds that increase phosphorous availability within soil (Harper & Pendelton 1993). Biocrusts occur in all dryland regions of the world and on every continent, including polar regions, and have been observed on nearly all soil types (Budel et al. 2003). Crusts are found in the spaces between and under vascular plants, and in some regions where persistence of biocrust communities can be disproportionately favored (hot deserts or cool/cold drylands), can occupy 70% or more of the living ground cover (Belnap 1995; Fig. 1.1a).

Although biocrusts have received significant attention in recent research in dryland ecosystems, bryophytes remain the poorest understood component of the community. In drylands where soil-dwelling mosses can persist, moss can account for 2% to over 30% cover in crusts (Thompson *et al.* 2005), often depending on crust maturity, and are a taxonomically diverse

functional group (Brinda et al. 2007). Many common crust moss genera (e.g. *Syntrichia, Tortula, Pterogonium, Crossodium, Didymodon*) belong to the family Pottiaceae, and are typically slow-growing acrocarps with annual (normally short mosses <0.5 mm in height) as well as perennial (0.5 – several cm in height) life history strategies (Rosentreter *et al.* 2007).

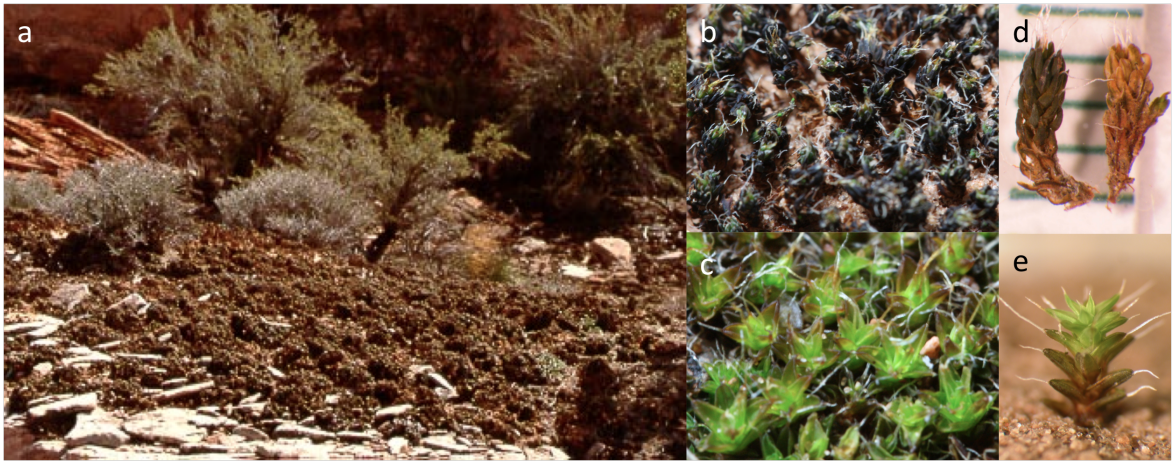


Figure 1.1: (a) Moss in an intact biocrust from the Colorado Plateau in the Western United States; (b) the common biocrust species *Syntrichia caninervis* at ~6x magnification in the desiccated state, and (c) in the hydrated state; (d) dry *S. caninervis* shoots exhibiting normal (left) and reduced (right) pigmentation as a result of rapid wet-dry cycles (photo credit: Lloyd Stark); and (e) architecture and new growth of an emerging *S. caninervis* shoot (photo credit: Lloyd Stark).

Evidence suggests that while mosses offer significant contributions to overall biocrust function, they can be sensitive to environmental alterations, from discrete soil disturbance events to direct and subtle aspects of climate change. Moss removal from a biocrust has negative consequences for structure and composition of the crust as well as nutrient cycling in soils below (Reed *et al.* 2012, in review). Therefore, understanding the physiological determinants of performance and survival of biocrust mosses is important for understanding dryland ecosystem ecology in general. Biocrust mosses possess a suite of adaptations to cope with environmental

variability in dryland systems, and the physiological ecology of dryland mosses has received recent research interest, mainly in terms of the response of these organisms to environmental change but also to the ecological role of moss as part of crust communities. This chapter will review the current knowledge of biocrust moss ecophysiology in dryland systems, and will concentrate on water relations, temperature and light tolerance, nutrient status, and responses to elevated CO_2 with respect to photosynthetic performance, reproduction, biomass accumulation, and stress tolerance.

Desiccation tolerance, precipitation pulses, and carbon balance

The growth environment of dryland biocrust mosses is often characterized by extended dry periods interspersed with small precipitation events, resulting in intermittent pulses of resource availability. This, along with high temperatures and exposed soil microclimates results in rapid drying and prolonged periods of desiccation. Because of these factors, there is thought to have been strong evolutionary pressure and selection for a desiccation tolerant strategy among biocrust species (Oliver et al. 2000; Oliver et al. 2005; Proctor et al. 2007).

All mosses are poikilohydric organisms where the water content of cells is in equilibrium with the environment and the control of tissue water content is passive. Moss gametophytes are often exposed to a range of environmental water availabilities, and as a consequence must possess the ability to withstand an extreme range of cellular water contents. In drylands, cell water content can often be very low for extended periods. Most, though not all, dryland mosses therefore display some degree of desiccation tolerance. Although cellular water potentials at full turgor are typically between -1.0 and -2.0 MPa, many mosses remain photosynthetically active

over water potentials ranging from -0.5 to -10.0 MPa (Dilks & Proctor 1979; Proctor 2008). The limits of tolerance for most mosses are cellular water potentials of -20 to -40 MPa (Proctor & Pence 2002; Oliver et al. 2005; Proctor et al. 2007); the degree of this tolerance depends largely on the growth environments that species are adapted. Mosses with the highest degrees of desiccation tolerance often represent the endpoints of these spectra. While highly desiccation-tolerant mosses occur in dry microclimates (on substrates such as exposed rock, sand, or bark) in all biomes, the diversity and abundance of such species is highest in dryland ecosystems.

Desiccation tolerance represents a strategy to suspend metabolism during dry intervals, and restrict physiological activity to periods of sufficient hydration. During desiccated periods, shoot tissues lose virtually all liquid water and can dry to 5-10% dry mass and water potentials of -100 MPa in the most desiccation tolerant species (Proctor et al. 2007; Fig 1.1b). A significant degree of molecular packaging mechanisms involving sugars (Smirnoff 1992) as well as proteins (Oliver *et al.* 2005, Buitink *et al.* 2002) are likely involved in the protection of macromolecules in the absence of water as well as maintenance of spatial relationships in cells. Mechanisms of antioxidant production to neutralize reactive oxygen species as well as photoprotection to dissipate excess light (Marschall & Proctor 2004) also appear to be important as cells transition to the desiccated state. Because it influences the degree to which tissues are preserved and energy is invested in preservation, the rate at which tissues dry is an important determinant of survival in the desiccated state as well as recovery potential for many species, and in general drying speed is inversely correlated with future performance (Oliver et al. 2000; Oliver et al. 2005). When compared to shorter dry times, numerous measures of regeneration potential such as cell ultrastructure, pigmentation, electrolyte efflux, and photosynthetic performance have all been shown to be higher in mosses exposed to longer drying periods (Schonbeck & Bewley 1981

a, b; Oliver & Bewley 1984; reviewed in Oliver et al. 2000). This relationship is due to the ability of cells to initiate and complete preservation procedures for macromolecules and organelles prior to entering the desiccated state. In general, the more preserved tissues are while desiccated, the higher their recovery potential is given sufficient hydration.

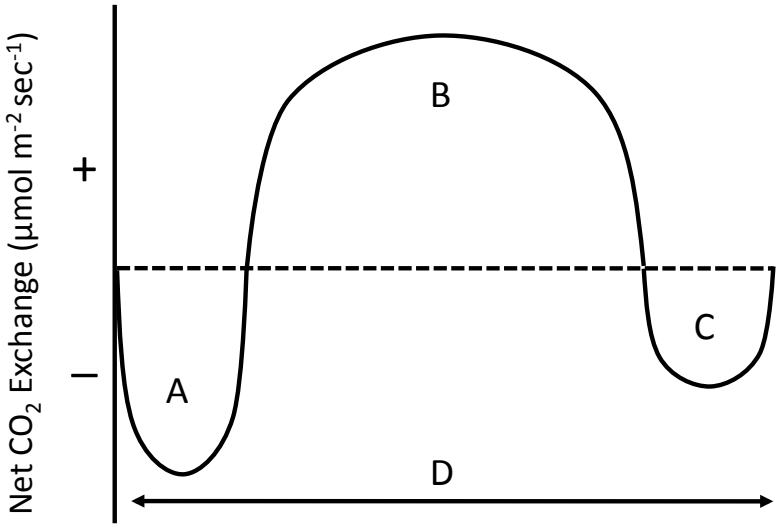


Figure 1.2: Characteristic pattern of net carbon fixation in desert moss over the course of a wet-dry cycle (of length D) initiated by a rainfall event: an initial period of net carbon loss when respiratory costs are high (A) followed by a period of net carbon gains when tissues are hydrated and photosynthesis is higher than respiration (B; length varies as a function of D) and a period of subsequent carbon loss as photosynthesis ceases and tissues prepare for desiccation again (C). Following an individual rainfall event, carbon balance is equivalent to gains from net carbon fixation (area under B) minus carbon loss from respiration (areas under A + C). (adapted from Coe et al. 2012b *Ecology*, also see Ch. 3 in this dissertation).

Among mosses, dryland species display the highest levels of desiccation tolerance recorded, both in terms of the length of time a shoot can remain desiccated before full physiological recovery as well as the lowest relative water content (RWC) from which tissues can recover. Dryland biocrust species can survive in the desiccated state (e.g., 5-10% dry mass in *Syntrichia*) for months and recover full photosynthetic function within <24 h (Tucker et al. 1975; Oliver et al. 1993; Proctor & Pence 2002). In the extreme, mosses have been observed to remain

desiccated for > 100 days in the Mojave Desert (Stark 2005). In the desiccated state, shoots can withstand significantly more variability in temperature and light levels, including those that can damage tissues when hydrated. Indeed, because of the stressful environments commonly occupied by biocrusts, many dryland crust mosses may actually *require* periods of desiccation for survival under temperature and light extremes.

From the perspective of a moss, when rainfall does occur in dryland systems it occurs as a pulse between two desiccation periods. This results in a wet-dry cycle and a characteristic response with an initial period of net carbon loss (where carbon loss through respiration is greater than carbon gain through photosynthetic fixation) and net carbon gain (when fixation outweighs respiration) (Fig. 1.2). Respiration recovers more rapidly than photosynthesis (due to a lag time in reinstatement of the Calvin cycle) thus the onset of hydration is characterized by a period of net carbon loss as respiratory energy is used to reinstate metabolism, repair membranes, and reconfigure and reorganize cellular components (Hinshiri & Proctor 1971; Tuba et al. 2004; Proctor et al. 2007). Because the photosynthetic apparatus remains intact during desiccation, recovery of photosynthetic function in biocrust mosses is often rapid (only slightly lagging behind respiration), and net carbon fixation can be reached in 10-30 minutes following hydration in some desiccation tolerant species (e.g. *Tortula (Syntrichia)* spp.; Bewley 1979; Tuba *et al.* 1996; Deltorro et al. 1998; Proctor & Smirnoff 2000; Reed et al. in review; Li et al. 2010). Once photosynthesis outpaces respiration, a phase of net carbon gain occurs, the length of which is determined by the duration of tissue hydration. As tissues dry, photosynthetic rates diminish, and as energy is used to repackage cellular contents for another bout of desiccation, the cycle often ends with another brief phase of net carbon loss (Mishler & Oliver 2009; Reed et al. in review). Depending on the relative magnitude of the periods of carbon gain and loss over the

course of the wet-dry cycle, dryland mosses exhibit either an overall carbon balance that is positive or negative. The carbon balance of biocrust mosses during discrete events, when compounded over long periods, is a strong determinant of performance, growth, and long-term survival. Interestingly, this phenomenon appears to apply to mesic mosses as well and has been shown to be the case in tropical bryophytes exposed to discrete hydration events.

Water Relations

In spite of passive water regulation over the gametophyte surface, *cell* water relations of mosses are nearly identically to that of other dryland plants with respect to physiological parameters such as water potential (ψ_w) and its relationship to RWC. As is true in mosses from other environments, the RWC of dryland biocrust species is a strong determinant of photosynthetic performance. Maximum rates of net carbon fixation are typically reached between 40 and 70% RWC (Tuba et al. 1996; Coe, unpublished) as photosynthesis is inhibited at higher water contents by limited CO_2 diffusion and at lower water contents by low intracellular ψ_w. However, many dryland mosses possess a greater range of suitable hydration levels for photosynthesis and can withstand cell water potentials (when not physiologically active) well below those of species from mesic environments. Some dryland species (e.g. *S. ruralis*) can withstand cellular drying to a ψ_w of <-100 Mpa when desiccated and photosynthesis and respiration have been detected in tissues with ψ_w as low as -10 and -20 Mpa, respectively (Dilks & Proctor 1979). Sustained physiological functioning at such water potentials possibly owes to higher cell wall thickness relative to lumen, high cell wall extensibility, and/or low RWC at full turgor (Proctor et al. 1998; Proctor & Tuba 2002).

Dryland mosses possess a suite of morphological and physiological adaptations to maximize photosynthetic performance under dry conditions as well as to take advantage of water when it does become available. At the macro-scale, biocrust species almost always grow within the laminar boundary layer directly above the soil surface, thus minimizing convective water loss. In dryland mosses, cuticular waxes deposited on leaf surfaces play a role in restricting water loss (Xu et al 2009), as do hair points at the ends of leaves (e.g. *Grimmia, Syntrichia*) that serve to extend the height of the boundary layer and increase albedo (Valko 2003; Bowker et al. 2010). Shoots of biocrust mosses are also typically short and densely packed in a characteristic clonal cushion or carpet habit, which behaves as a smooth object on the soil surface when exposed to wind (Proctor 2000), and maximizes extracellular water storage between adjacent shoots in capillary spaces.

Capillary water is an essential component of the physiological ecology of dryland mosses for several reasons. First, although some water does move through cells and cell walls, most water conduction is external and water held or moving within capillary spaces between shoots, in sheathing leaf bases and in rhizoid tomenta acts as a direct source of water for the plant. Second, capillary water can be a major component of the water associated with a shoot, can outweigh symplastic water by a factor of five or more (Proctor 2008), and can thus act as a primary source of water storage for moss. Indeed, water stored in this manner is directly related to the length of time shoots remain wet and photosynthetically active and can often assist dryland moss species in bridging gaps in water availability by extending the hydrated period provided by a rain event. Finally, capillary water content can vary widely without influencing the water status of plant cells in a turgid state, but when it is exhausted, cells dry rapidly. One hypothesized function of capillary water in *Tortula ruralis* is to maintain cells at full turgor until a cytological switch

initiates rapid desiccation, whereby cells spend little time at intermediate water contents thought to impose damage (Gaff 1997; Proctor 2000). This hypothesis has not been fully explored in different species, however, and may conflict with the widely demonstrated relationship between drying speed and recovery potential.

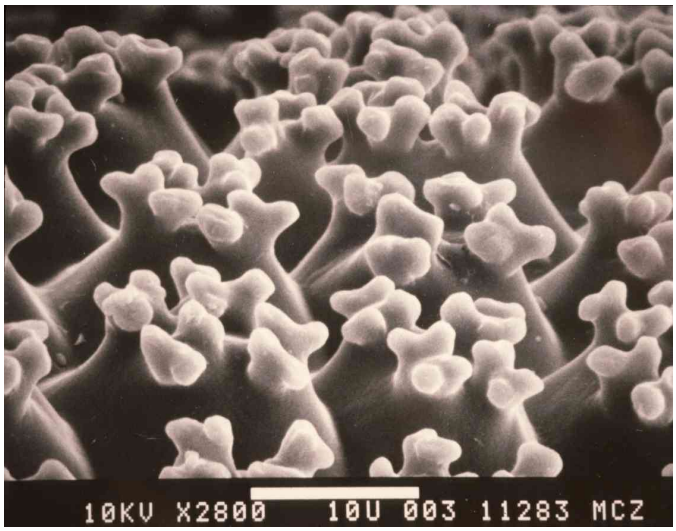


Figure 1.3: Scanning electron micrograph of papillae on the surface of cells in *Syntrichia*: when cell surfaces are wet, carbon fixation occurs in the tips of papillae where chloroplasts are concentrated, while interstices serve as external conducting channels for water. (Photo credit: Brent Mishler).

Mosses must reconcile maximizing water conduction and storage and the need for gas exchange between the atmosphere and the interior of the leaf for photosynthesis. When shoots possess external water, exchange of CO_2 must occur through films of water where diffusion resistance for gas is four orders of magnitude higher than through air. Typical adaptations to minimize such trade-offs include specialized leaves that perform either water conduction *or* gas exchange or the use of the outer surfaces of stem-sheathing leaves for gas exchange while employing inner surfaces to provide a capillary channel around the stem (Proctor 2008). Some

additional adaptations are present in dryland genera and often involve the division of water conducting and gas exchange surfaces in the same structure or section of leaf on microscopic scales. For example, the genus *Syntrichia (Tortula)* is characterized by specialized papillae or mammillae extruding from the cellular surface (Fig. 1.3) that serve two simultaneous functions: first, to create a continuous network of interstices for water adhesion and transport in microcapillary spaces between papillae, and, second, to allow sustained photosynthesis at high water contents in the tips of papillae (also shown to contain proportionally higher chloroplast densities) even when the rest of cells are covered with a sheet of water that impedes CO_2 diffusion (Tucker et al. 1974; Proctor 1979a; Xu et al. 2009).

Irrespective of adaptations to limit water loss in dryland mosses, overall environmental water availability (primarily through alterations in precipitation regime) is likely to influence performance and survival. This is because such alterations result in changes in carbon balance following rainfall events, and biocrust mosses are dependent on the maintenance of positive carbon balances from discrete rainfall events for biomass accumulation and long-term viability. Much of the recent research on this topic has been conducted in western North America, where climate models predict changes in mean annual rainfall as well as alterations in intra-annual precipitation parameters including individual event magnitude, timing (frequency) at which events fall, and the time of year precipitation occurs (Lioubimtseva et al. 2004; Meehl et al. 2007; Seager et al. 2010). Rainfall event magnitude is one of the largest determinants of carbon balance in the common biocrust species *Syntrichia caninervis*, because increased rainfall amounts result in shoots that remain hydrated (thus remain in the phase of net carbon fixation; Fig. 1.2) longer, and small events result in limited gains from carbon fixation that cannot compensate for respiratory costs during the event (Barker et al. 2005; Coe *et al.* 2012a; Reed *et*

al. in review). Recovery of the cell cycle as well as reassembly of cytoskeletal elements can take >24 hr in *Syntrichia*, thus brief periods of hydration may not be sufficient for full recovery of physiological function. Further, several studies indicate a rainfall event size threshold for carbon balance in dryland mosses of 2-3 mm, below which moss will, on average, enter carbon deficit (Fig. 1.1d; Stark 2005; Barker et al. 2005; Coe et al. 2012a). Given that >70% of rainfall events in drylands are <5 mm effective size (Sala & Lauenroth 1982; Loik et al. 2004; Reynolds et al. 2004) and within that, the majority are less than half of that (Huxman et al. 2004), it is probable that small carbon deficits are quite common and dryland mosses are reliant on the presence of larger events for net carbon gain. The frequency with which events fall also influences carbon balance, and Coe *et al.* (in press) suggest that increasing the dry interval between events reduces carbon balance during subsequent events. This likely results from increased cost of recovery during hydration following periods of increased desiccation intensity (Hinshiri & Proctor 1971; Dilks & Proctor 1974; Proctor & Pence 2002; Proctor 2003).

Most dryland biocrust species depend on precipitation during the cooler months of the year for annual growth because ambient temperature and humidity permit water from rainfall events to remain available for sustained periods. During the warmer months, meteorological conditions restrict water availability for biocrust species, and mosses appear to exhibit changes in physiological state over the course of the year that influence response to rainfall. For example, when measured under identical laboratory conditions, mosses collected in the winter display higher responsiveness to rainfall and higher average carbon balances when given same event size that caused significantly smaller carbon balances (and sometimes loss) compared to those collected in the summer (Coe et al. 2012a). Several climate models for North American drylands suggest winter rainfall will be reduced, while summer will exhibit more frequent small events

and/or an increase in monsoonal systems (Castro et al. 2001; Meehl et al. 2007). The consequences of such changes could be severe, and it has been suggested that (a) more frequent < 2 mm events during the summer may cause rapid moss decline and reduced nitrogen availability to crust organisms (Reed et al. in review) and (b) an increase in monsoonal activity could have negative consequences for sex expression and associations with nitrogen fixing cyanobacteria in *Syntrichia* (Stark et al. 2011). Finally, reductions in winter rainfall will be detrimental to many biocrust mosses that rely on cumulative carbon gains during these times of year to compensate for carbon losses during warmer, unfavorable periods.

Temperature relations

In dryland systems, air temperatures have wide diurnal and annual fluctuations, biocrust mosses grow in close proximity to the soil surface, and shoot temperatures can exceed air temperature by 19 °C or more (Hearnshaw & Proctor 1982). Under these conditions, selective pressures to tolerate such extremes are high, and as a consequence, the range of temperature under which dryland biocrust mosses can survive greatly exceeds that of species from other biomes. Survival in the desiccated state in these species has been noted in temperatures ranging from <0 °C (for months) up to 100 °C (for minutes) (Hearnshaw & Proctor 1982) and dry biocrust mosses on sun-exposed substrates can frequently reach tissue temperatures >60 °C (Proctor 2010). The ability to withstand high temperatures in the desiccated state in particular contributes to the ability of dryland mosses to colonize and persist in unfavorable microclimates from which other less-tolerant species may be excluded (Kidron et al. 2000). Most biocrust species have perennial gametophytes, thus viability while desiccated enables shoots to persist

throughout the entire year and bridge gaps in hydration occurring during the warmer months where growth cannot occur.

Temperature tolerance while hydrated, however, in biocrust moss taxa is similar to the range of many other bryophytes. Hydrated shoots are far less tolerant of temperature extremes, and tissue damage occurs at both the high and low ends of the temperature spectrum. When shoots are moist, tissue injury generally begins to occur when temperatures exceed 40 °C (Larcher 2003), with lethal high temperatures between 42-51 °C, depending on species and exposure time (Meyer & Santarius 1998; Proctor & Pence 2002). Less is known about responses to cold temperatures and although many dryland biocrust species survive and actively photosynthesize and grow throughout the winter, freezing (< -10 °C) of hydrated shoots may cause irreversible photoinhibition if repair mechanisms are not present (Lovelock et al. 1995). Production of isoprene (2-methyl-1, 3 butadiene) under conditions of temperature stress has been shown to have protective functions in some mosses that experience wide temperature fluctuations in nature (Hanson et al. 1999). Although thermoprotection via isoprene emission has not yet been demonstrated in desert mosses, evidence for the evolution of its function in several early land plant lineages (Sharkey & Yeh 2001; Jobson & Qiu 2011) suggests it is worth considering its potential presence in this functional group.

Temperature exerts strong control on net carbon fixation in dryland species because of (a) biochemical limitations such as compromised membrane stability and enzyme denaturation, (b) increased respiration rates, and (c) reductions in environmental water availability due to increased rates of evaporation. Optimal temperatures for photosynthesis occur between 10-20 °C (Furness et al. 1982; Alpert & Oechel 1987; Coe, unpublished data), and suppression of photosynthesis has been observed to occur at temperatures between 25 and 35 °C, depending on

geographic locality and crust moss species (Grote *et al.* 2010; Coe *et al.* 2012b). At these temperatures, declines have been observed in net photosynthesis (Grote *et al.* 2010), carboxylation capacity, maximum rates of electron transport through photosynthetic membranes (Coe et al. 2012b), and efficiency of conversion of light energy to chemical energy (Hamerlynck et al. 2002, Coe *et al.* 2012b). Declines in photosynthetic performance are likely due to induced structural deficiencies such as enzyme denaturation, damage to chloroplast membranes and damage to the photosynthetic pigment apparatus (Larcher 2003), changes in membrane permeability associated with high heat while hydrated (Meyer & Santarius 1998; Liu *et al.* 2003), and/or offsets in the optimal temperature for photosynthesis as compared to respiration (Grote *et al* 2010).

Because carbon fixation in dryland mosses is constrained at high temperatures, limited energy is available for physiological functioning, growth, stress tolerance, and reproduction. As a consequence, mosses growing in environments characterized by thermal stress are faced with a corresponding suite of energetic trade-offs. Male and female shoots in many biocrust moss taxa differ by an order of magnitude in energetic allocation to reproductive structures (Lackner 1939; Paolillo 1979; Bowker et al. 2000); based on dry biomass, antheridia cost approximately six times as much as archegonia to produce (Stark et al. 2000). Therefore, male shoots are constrained by the cost of their reproductive structures, and in stressful conditions, are forced to reduce proportional carbon allocation to growth or stress tolerance. Females, on the other hand, can allocate proportionally more carbon to growth and stress tolerance while continuing to produce reproductive structures. To illustrate, leaf growth and regeneration in female shoots occurred twice as fast and was more complete than in males exposed to incremental increases in temperature from 25 to 45 °C for 60 minutes (Stark & McLetchie 2006). These differences cause

overall differences in stress tolerance abilities in male and female gametophytes, and have resulted in two common patterns in the field. First, dryland mosses ehbitit sex ratios in nature that differ substantially from 1:1. Ratios are almost always female biased, with male shoots occurring <30% of the time (or as low as 0%) at the population level (Stark & McLetchie 2006). Such female-skewed ratios are commonly observed in *Bryum* (Stark *et al.* 2010), *Didymodon* (Ochyra & Zander 2007), *Syntrichia* (Mishler & Oliver 1991; Stark et al. 2005), and in an array of biocrust species from the Mojave Desert in North America (Brinda *et al.* 2007). Such patterns are directly related to the inability of males to grow and produce reproductive parts in environments characterized by thermal stress (Stark & McLetchie 2006). Second, dryland mosses exhibit spatial segregation of the sexes (SSS) based on gradients of environmental stress, where females are more common in thermally stressed environments such as plant interspaces, and often males are restricted to shrub understories (Bowker et al. 2000; Stark *et al.* 2005; Stark *et al.* 2010).

When female shoots do produce sporophytes (though it is very uncommon in dryland species due to SSS and low water availability for sperm transfer to archegonia), the cost of production and maintenance of these structures is much higher than production of archegonia alone. Abortion of sporophytes is an extremely common occurrence in dryland biocrust taxa for two reasons. First, the energetic cost of maintaining a sporophyte (due to investment of structural materials as well as maintenance of cellular machinery) is high, and places additional stress on the female gametophyte (Stark et al. 2000; Stark 2001). Second, sporophytes are less tolerant of thermal stress than gametophytes (illustrated in *Microbryum* heat shock experiments; McLetchie & Stark 2006). An understanding of the steps leading to sporophyte abortion is confounded by the fact that the sporophytes are connected to and dependent on gametophytes, making

mechanistic inferences into the process and reasons for abortion difficult. Nonetheless, female shoots appear to be more stress tolerant and possess higher growth rates than males under conditions of thermal stress, unless they produce sporophytes.

In dryland systems, thermal stress, skewed sex ratios, and SSS result in rates of sexual reproduction that are low to absent (Bowker et al. 2000). Although high surface temperatures and large variation in temperatures impose significant amounts of stress on carbon acquisition and physiological functioning, environmental correlates of temperature (such as water and nutrient availability or light levels) along environmental gradients can play a large role in biocrust moss physiological ecology and reproductive biology. For example, water availability is shown to correlate with temperature along a canopy – interspace gradient (Stark *et al.* 2010), and has been shown to influence sex expression in *S. caninervis* due to males' inability to tolerate energy loss associated with repeated wet-dry cycles in plant interspace regions (Benassi *et al.* 2011).

Response to variation in light

Bryophytes as a group have high variation in light responses as well as seasonal and plastic variability (Proctor 2000). Dryland crust mosses may represent some of the extremes in plasticity of response as they typically occur in plant interspaces that experience high light conditions (PAR > 1000 $\mu molm^{-2} sec^{-1}$), yet also can occupy habitats directly under low desert shrubs. When biocrust mosses are desiccated (and not physiologically active), they display higher tolerance of high light than wet shoots (Seel et al. 1992). Mosses that can persist in areas where they are exposed to extreme conditions (e.g. high irradiance plant interspaces) often, but not always, do so because they can survive in the desiccated state when the growth environment

is extreme. Biocrust species are not always dry under conditions of high irradiance though, particularly following short rainstorms, thus still must be able to perform photosynthetically in and tolerate high light conditions while hydrated.

Most mosses fall into the category of shade plants based on their photosynthetic physiology (Valanne 1984). Their photosynthetic apparatuses saturate at low irradiances, and express low chlorophyll a:b ratios (Martin & Churchill 1982; Marshall & Proctor 2004). Some biocrust taxa (e.g. *Bryum*) fall into this category and are often restricted to crusts under significant plant cover. Such mosses growing in lower light environments such as under shrubs exhibit higher photosynthetic rates at lower irradiances than those growing in exposed environments (Alpert & Oechel 1987). Further, even in biocrust taxa, photosynthetic activity typically occurs under <20% full sun following rainfall events where cloud cover reduces PAR to 50-250 μmol m^{-2} sec^{-1} (Marschall & Proctor 2004). Some of the more common species (e.g. *Syntrichia ruralis*, *S. caninervis* , *Rhacometrium spp.*), however, saturate at higher irradiance levels. In open sun environments, *S. ruralis* exhibits 95% saturation at PAR levels of 832 – 935 μmol m^{-2} sec^{-1} and full saturation of *S. ruralis* (Proctor 2008) as well as *S. caninervis* (Coe, unpublished) occur at PAR levels >1000 μmol m^{-2} sec^{-1}.

Adaptations to protect tissues from excess light energy that are present in dryland biocrust mosses include photoprotection as well as energy dissipation mechanisms, though there is still some debate as to which mechanism is more important in these species (see Marshall & Proctor 2004). Photoprotection by xanthophyll cycle carotenoids to scavenge free radicals appears to be important in both dry and wet tissues (Hamerlynck et al. 2002; Marschall & Proctor 2004). Energy dissipation via non-photochemical quenching (NPQ) has been estimated using chlorophyll fluorescence techniques, and appears to also play an important role in response

to high light. When exposed to irradiances >1000 μmol m^{-2} sec^{-1}, *S. ruralis* displays NPQ levels that exceed that of more mesic species (Proctor 2000), and sun-exposed desiccation tolerant species tend to display NPQ values >2 times higher than more mesic mosses (Proctor & Smirnoff 2000; Hamerlynck et al. 2002; Proctor & Smirnoff 2010). However, NPQ levels diminish rapidly following several minutes in the dark and appear to be suppressed by violaxanthan inhibitors (Proctor & Smirnoff 2000). The current body of work suggests that photoprotection in biocrust mosses include strategies to dissipate excess energy as heat and regulation of xanthophyll-mediated protection appears to act as a control point that governs overall responses.

Responses to elevated CO_2

For all photosynthetic organisms, the concentration of CO_2 at sites of carboxylation and, hence, responses to changes in CO_2 concentration, depends upon the atmospheric CO_2 concentration and diffusion rates to the point of carboxylation. Mosses differ from more commonly studied higher plants in that the diffusional pathway does not include stomata and is very sensitive to the saturation level of tissues in the diffusion path. However, biocrust mosses are similar to higher plants in that they are both responsive to both short-term and long-term elevated CO_2 treatments, and responses have been studied in experiments that include laboratory fumigation treatments in chambers, open-top chambers in the field, and free air CO_2 enrichment (FACE) rings.

Biocrust mosses display CO_2 compensation points (the CO_2 concentration at which net carbon assimilation becomes positive) that fall within the typical range for C_3 plants (Dilks

1976), and short-term (<1 year) exposure to elevated CO_2 can result in a stimulation of CO_2 uptake by 30% or more (Tuba *et al.* 1998) because of increased substrate for photosynthesis. Assimilation rates in *S. ruralis* were shown to increase by 30-35% after initial exposure and remain significantly (~20%) higher than ambient-grown mosses after growth for five months in a CO_2 enriched environment (700 ppm; Tuba et al. 1998), yet starch and sugar content remain unchanged compared to ambient-grown mosses after this length of time (Csintalan et al. 1997). This suggests that photosynthesis is stimulated in the short term, that photosynthetic acclimation may not occur within the first year of fumigation, and that additional assimilated carbon is immediately used for energy rather than stored.

Following longer term (>1 year) exposure to elevated CO_2, carbon content of shoots is altered in biocrust moss, and this change appears to influence physiological trade offs among allocation to growth, sex expression, and stress tolerance. Research on *S. caninervis* collected from the Nevada FACE facility after 10 years exposure to CO_2 enrichment has shown that increased photosynthetic efficiency results in increased percent carbon per unit mass of shoots (Brinda *et al.* 2011; Coe *et al.* 2012b), but it is also appears that this does not always translate into increased shoot growth, and shoot growth in elevated CO_2 can even be significantly shorter compared to ambient-grown shoots if they express sex (Brinda *et al.* 2011). This may be because dryland biocrust species such as *S. caninervis* are slow growing stress tolerant species where growth is often sacrificed for other physiological processes, thus they may not respond to CO_2 enrichment with a marked increase in structural biomass, and fixed carbon may be allocated to physiological functions or energetic requirements other than growth. Further, in arid systems, larger plants may even be at a disadvantage due to higher rates of moisture loss.

It is currently unclear if biocrust mosses will acclimate photosynthetically to long-term CO_2 enrichment. Several studies have suggested that photosynthesis is downregulated after long-term exposure (Otvos & Tuba 2005), and that reductions in Rubisco may occur (Long et al. 2004). However, Coe et al. (2012b) demonstrate that mosses after 10 years of CO_2 enrichment display higher photosynthetic rates at field growth concentrations and no reductions in either supply of CO_2 to carboxylation sites or rates of RuBP regeneration. The results from the Coe et al. study suggest that, because bryophytes possess neither the capacity to alter CO_2 supply via stomatal conductance nor the limitations imposed by reduced sink strength in phloem transport, photosynthetic performance remains unaltered in the long term.

Carbon allocation patterns do appear to change after long-term elevated CO_2 exposure, and may influence the tolerance of environmental stress and sex expression. Desiccation tolerance appears to be enhanced in biocrust moss grown in elevated CO_2 and likely mechanisms include: (a) increased protection of cellular components with sugars and starch while in the desiccated state; and/or (b) increased regeneration potential of protonema following a desiccation event (Brinda *et al.* 2011) due to allocation of stored energy reserves to repair or to cellular processes that work to mitigate damage due to desiccation.

Growth in elevated CO_2 appears to influence sex expression in biocrust species. Brinda *et al.* (2011) found that compared to shoots grown in ambient conditions, *S. caninervis* exposed to 10 years of CO_2 enrichment displayed accelerated sexual maturation, and sex expression in shoots was twice as likely.

The thermotolerance of photosynthesis in biocrust mosses appears to be enhanced by long-term exposure to elevated CO_2, and *Syntrichia* grown in elevated CO_2 and exposed to high (35-40 °C) temperatures exhibited increased CO_2 assimilation (Proctor 1982), increased

conversion efficiency of light energy into chemical energy in the photosynthetic light reactions, increased electron transport rates during photosynthesis, and increased availability of CO_2 at sites of carboxylation as compared to shoots grown at ambient CO_2 (Coe *et al.* 2012b). Photosynthetic thermotolerance can be enhanced by a number of means, and two commonly cited mechanisms are enhanced membrane stability at high temperatures and increased Rubisco activase activity under stress (Sharkey et al. 2001; Sharkey & Schrader 2006). Either or both of these possibilities could account for elevated CO_2-induced thermotolerance in desert mosses. Based on estimates of electron transport efficiency and diffusion of CO_2 through photosynthetic membranes, Coe et al. (2012b) suggest enhanced membrane stability is the most parsimonious explanation for thermotolerance in *S. caninervis* exposed to elevated CO_2 for 10 years. The role of Rubisco activase, however, in thermotolerance of biocrust mosses has received very little attention, yet could be equally important in protecting photosynthesis under high heat conditions. It is probable, based on work in other dryland plants (Sharkey et al. 2001) that increased amounts of Rubisco activase as a function of elevated CO_2 exposure could lead to less diminished photosynthetic rates under high temperatures. In spite of multiple possible mechanisms, elevated CO_2-induced photosynthetic thermotolerance is particularly important for biocrust mosses as shoots are exposed to a wide range of temperatures in dryland systems and are likely to experience increasingly heightened extremes in temperature in the future (Meehl et al. 2007).

In sum, stress tolerance is often favored at the expense of growth for dryland biocrust mosses, and elevated CO_2 appears to accentuate this trade-off. Carbon supplementation influences allocation to growth, stress tolerance, and sex expression in many species, and excess carbon from a more efficient photosynthetic process is preferentially allocated towards processes such as thermotolerance of photosynthesis and desiccation tolerance of shoots, although

productivity and biomass may not change (or even decline) under CO_2 enrichment. This suggests that although growth rates may become even slower in these already slow-growing plants, biocrust mosses may be among the plants that exhibit sustained persistence in a CO_2-enriched future atmosphere in dryland systems due to enhanced performance under environmental stress.

Nutrient relations

Second only to water, nitrogen often strongly limits primary production in arid regions (Vitousek & Howarth 1991; Smith et al. 1997). Mosses (including biocrust species) primarily receive nutrients from atmospheric deposition (Bates 2000) and do not rely on uptake from soil. Therefore, they are often buffered from belowground nutrient limitation and increased nitrogen deposition from atmospheric sources is likely to influence productivity of dryland mosses and their relative abundance in crusts. Because biocrusts influence cycling of trace elements and are important in regional and global budgets (Zaady et al. 2000), such effects may extend to influence ecosystem biogeochemistry.

Nitrogen appears to be a limiting factor for certain aspects of reproductive biology in crust mosses. Sex expression in *S. caninervis* has been shown to be stimulated following four years fertilization with 10 kg N ha^{-1} yr^{-1} and then suppressed under a higher treatment of 40 kg N ha^{-1} yr^{-1} (Stark et al. 2011) suggesting that reproduction is nitrogen limited, but excess levels can have detrimental effects. In this same study, productivity and regeneration vigor were unaffected by the high nitrogen treatment, suggesting some growth parameters were decoupled from environmental nitrogen levels. At larger spatial scales, increased nitrogen to mosses appears to

alter certain ecological dynamics due to interactions with other members of dryland communities. In general, exposure to supplemental nitrogen has a negative fertilization effect on mosses due to tissue toxicity or competitive exclusion by vascular plants (van der Wal et al. 2005), and the latter effect has been partially implicated in facilitation of Cheatgrass (*Bromus tectorum*) invasions in western North America (Schwinning et al. 2005). Finally, there may be variation in levels of cyanosymbiosis in dryland biocrust species (with the cyanobacterial genera *Microcoleus* and/or *Nostoc*) under changes in nitrogen availability. Changes in levels of such associations are likely to influence nitrogen fixation rates and soil fertility on ecosystem scales.

It is important to point out that while nitrogen availability plays a role in certain ecophysiological responses of biocrust moss, water availability overwhelms these effects under most circumstances. Experiments that have altered water availability and nitrogen levels simultaneously (e.g. Stark et al. 2011, and ongoing work in the Mojave Desert) show that productivity and sex expression are influenced to a greater degree by water than nitrogen. Additionally, compared to hydrated controls, intermittent hydration periods resulting in repeated wet-dry cycles reduces nitrogen uptake potential in crust moss (Bates 1997), suggesting that water relations and desiccation tolerance at the shoot level also influence response to environmental nitrogen levels.

Though nitrogen may be the most limiting nutrient for biocrust mosses, other elements such as phosphorus, potassium, and trace metals (Mg, Na, Ca, and Mo) appear to place constraints on growth and development (Belnap et al. 2001; Bowker et al. 2008). Atmospheric dust inputs significantly increase deposition of all of these bio-essential nutrients in Western North America (Belnap et al. 2001; Reynolds et al. 2001) and consequently enrich biocrusts and underlying soils (Belnap 2003). Mosses increase the surface roughness of biocrusts, and enhance

their capacity to capture dust (Belnap 2003; Fig. 1.4). Augmented dust inputs due to moss presence thus may serve to (a) enrich moss through direct uptake of deposited nutrients onto shoots, and (b) enrich crusts and soil from throughfall and enhanced nutrient turnover.

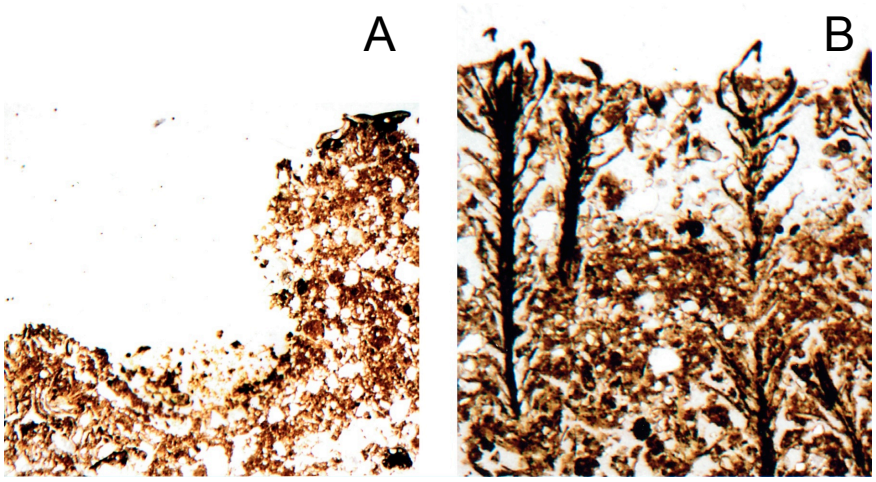


Figure 1.4: Sections through intact biocrusts reveal the relative ability of crusts without mosses (A) and with mosses (B) to capture atmospheric dust and particulate matter, which often serve as an important nutrient source in drylands. Photo credit: Belnap, J.

Distributions and ecological roles of biocrust moss in a future climate

Biocrust mosses influence crust structure and function through their effects on surface texture and nutrient cycling. Climate change and physiology are likely to interact to affect these ecological roles of mosses in dryland systems. Mosses control carbon flux into crusts and soil through CO_2 fixation, and photosynthetic rates are influenced primarily by water availability, but also by changes in CO_2 concentration and temperature. Carbon cycling in crusts will probably be influenced on shorter (1-5 year) timescales by alterations in the magnitude and timing of precipitation, and on longer (>5 year) timescales by concomitant increases in average temperatures and atmospheric CO_2. Nitrogen cycling in dryland crusts and soils is likely to be

influenced by foliar uptake by moss shoots and the degree of cyanosymbiosis present, both of which will be influenced by future atmospheric deposition rates. Yet perhaps more importantly, the presence of moss influences microclimate biogeochemistry, and has been shown to control belowground nitrogen availability and cycling within crusts (Reed et al. in review). Changes in biomass and moss percent cover due to alterations in intra-annual precipitation parameters may therefore exert more control on nitrogen cycling in aridlands compared to shoot-level physiological changes alone.

Distributions of dryland mosses are likely to be altered under future climate scenarios as well. Increases in average surface temperatures and in the frequency of extreme temperature events will place restrictions on suitable microhabitats of mosses within biocrusts and may exacerbate limited sexual reproduction due to spatial separation of the sexes. Rates of net carbon fixation and growth are likely to be suppressed under increased temperatures, limiting distributions in sun-exposed areas, yet elevated CO_2 may offset these limitations by enhancing stress tolerance. Finally, sun-exposed microhabitats that intensify water stress and reduce effective rainfall event size due to evaporation are likely to show reduced moss growth and establishment of young shoots, and may show some degree of replacement over time by habitats under shrubs where water and nutrient availabilities are higher.

Conclusion

Evolutionary pressure in desert ecosystems favors stress tolerance at the expense of growth for many organisms, and many of the adaptations present in biocrust moss and ecophysiological responses to environmental alterations reflect this trade-off. Owing to their

growth environment, dryland biocrust mosses are generally small, clonally growing species with a high level of desiccation tolerance. This strategy ensures that tissues can be protected from temperature and light extremes during extended dry periods, and restricts periods of physiological function, photosynthesis, and growth to intermittent periods of hydration. Changes in intra-annual precipitation patterns will likely heavily influence performance of dryland mosses because of changes in carbon balance, which when compounded over long time scales has a large influence on survival and presence within crusts. Elevated CO_2 is likely to interact with changes in temperature and rainfall to influence future performance in biocrust mosses by favoring stress tolerance and some aspects of sexual reproduction at the expense of growth.

As biocrusts are important in dryland regions for nutrient cycling and soil stability, and mosses play a key role in crust structure and function, the current body of knowledge contributing to our understanding of the physiological ecology of biocrust moss is essential in future ecosystem process modeling in dryland systems. We currently have little knowledge on cyanosymbiosis and its relation to nutrient status in dryland biocrust species, and only have limited information on the influences of CO_2 on time scales longer than one year, thus future work should focus on these aspects of crust moss ecophysiology.

References

Alpert, P. (2000). The discovery, scope, and puzzle of desiccation tolerance in plants. Plant Ecology*, 151* (1), 5-17.

Alpert, P., & Oechel, W. (1987). Comparative patterns of net photosynthesis in an assemblage of mosses with contrasting microdistributions. American Journal of Botany , 1787-1796.

Barker, D., Stark, L., Zimpfer, J., Mcletchie, N., & Smith, S. (2005). Evidence of drought-induced stress on biotic crust moss in the Mojave Desert. Plant, Cell & Environment , 28 (7), 939-947.

Bates, J. (1997). Effects of intermittent desiccation on nutrient economy and growth of two ecologically contrasted mosses. Annals of botany , *79* (3), 299-309.

Belnap, J. (1995). Surface disturbances: their role in accelerating desertification. Environmental Monitoring and Assessment , 37, 39-57

Belnap, J. (2003). The world at your feet: desert biological soil crusts. Frontiers in Ecology and the Environment *, 1* (4), 181-189.

Belnap, J., & Lange, O. (2001). Structure and functioning of biological soil crusts: a synthesis. Biological soil crusts: structure, function, and management , 471-479.

Belnap, J., & Phillips, S. (2001). Soil biota in an ungrazed grassland: response to annual grass (Bromus tectorum) invasion. Ecological Applications , 11 (5), 1261-1275.

Belnap, J., Phillips, S., & Miller, M. (2004). Response of desert biological soil crusts to alterations in precipitation frequency. Oecologia *, 141* (2), 1-11.

Belnap, J., Phillips, S., & Troxler, T. (2006). Soil lichen and moss cover and species richness can be highly dynamic: The effects of invasion by the annual exotic grass *Bromus tectorum*, precipitation, and temperature on biological soil crusts in SE Utah. Applied Soil Ecology , 32 (1), 63-76.

Benassi, M., Stark, L., Brinda, J., Mcletchie, D., Bonine, M., & Mishler, B. (2011). Plant size, sex expression and sexual reproduction along an elevation gradient in a desert moss. The Bryologist , 114 (2), 277-288.

Bewley, J.D. 1979. Physiological aspects of desiccation tolerance. Annual review of plant physiology Vol. 30, 1, 1979, p. 195

Bowker, M., Stark, L., Mcletchie, D. N., & Mishler, B. (2000). Sex expression, skewed sex ratios, and microhabitat distribution in the dioecious desert moss *Syntrichia caninervis* (Pottiaceae). American Journal of Botany 87(4): 517-526

Bowker, M., Belnap, J., Davidson, D., & Phillips, S. (2005). Evidence for micronutrient limitation of biological soil crusts: importance to arid-lands restoration. Ecological Applications , 15 (6), 1941-1951.

Bowker, M., Maestre, F., & Escolar, C. (2010). Biological crusts as a model system for examining the biodiversity-ecosystem function relationship in soils. Soil Biology and Biochemistry , 42 (3), 405-417.

Brinda, J., Stark, L., Shevock, J., & Spence, J. (2007). An annotated checklist of the bryophytes of Nevada, with notes on collecting history in the state. The Bryologist *, 110* (4), 673-705.

Brinda, J. C., Fernando, C., and Stark, L.R. 2011. Ecology of bryophytes in Mojave Desert biological soil crusts: Effects of elevated CO_2 on sex expression, stress tolerance, and productivity in the moss *Syntrichia caninervis* Mitt. In Tuba, Z., Slack, N. & Stark L.R. (eds.) Bryophyte Ecology and Climate Change. Cambridge Univ. Press.

Buitink, J., Hoekstra, F.A., and Leprince, O. 2002. Biochemistry and biophysics of tolerance systems. In Desiccation and survival in plants: drying without dying. Black, M. & Pritchard, H.W., Eds.

Castro, C., McKee, T., & Pielke Sr, R. (2010). The relationship of the North American monsoon to tropical and North Pacific sea surface temperatures as revealed by observational analyses. Journal of Climate, 14, 4449-4473.

Chopra, R., & Rahbar, K. (1982). Temperature, light and nutritional requirements for gametangial induction in the moss *Bartramidula bartramioides*. New Phytologist *, 92* (2), 251-257.

Clair, L., & Johansen, J. (1993). Introduction to the symposium on soil crust communities. Western North American Naturalist *, 53* (1), 1-4.

Coe, K.K., Belnap, J. & Sparks, J.P. (2012 a) Precipitation-driven carbon balance controls survivorship of desert biocrust mosses. Ecology (in press)

Coe, K.K., Belnap, J., Grote, E. & Sparks, J. P. (2012 b) Physiological ecology of the desert moss *Syntrichia caninervis* after ten years exposure to elevated CO_2: evidence for enhanced photosynthetic thermotolerance. Physiologia Plantarum. doi: 10.1111/j.1399-3054.2012.01566.x

Csintalan, Z., Takacs, Z., Tuba, Z. et al. 1997. Desiccation tolerance grassland cryptograms under elevated CO_2: preliminary findings. Abstracta Botanica 21: 309-15

Csintalan, Z., Proctor, M., & Tuba, Z. (1999). Chlorophyll fluorescence during drying and rehydration in the mosses *Rhytidiadelphus loreus* (Hedw.) Warnst., *Anomodon viticulosus* (Hedw.) Hook. & Tayl. and *Grimmia pulvinata* (Hedw.) Sm. Annals of Botany , 84 (2), 235.

Dilks, T.J.K. & Proctor, M.C.F. 1974. The pattern of recovery of bryophytes after desiccation. Journal of Bryology 8: 97-115

Dilks, T. (1976). Measurement of the Carbon Dioxide Compensation Point and the Rate of Loss of $^{14}CO_2$ in the Light and Dark in Some Bryophytes. Journal of Experimental Botany, 27(96), 98-104

Dilks, T., & Proctor, M. (1979). Photosynthesis, respiration and water content in bryophytes. New Phytologist , 97-114.

Evans, R., & Belnap, J. (1999). Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. Ecology *, 80* (1), 150-160.

Furness, S., & Grime, J. (1982). Growth rate and temperature responses in bryophytes: II. A comparative study of species of contrasted ecology. The Journal of Ecology , 525-536.

Gaff, D.F. 1997. Mechanisms of desiccation tolerance in resurrection vascular plants. In Mechanisms of environmental stress resistance in plants. pp. 43–58. In: Basra, A. S. & Basra, R. K. (eds), Mechanisms of environmental stress resistance in plants. Harwood Academic Publishers, London.

Grote, E., Belnap, J., Housman, D., & Sparks, J. (2010). Carbon exchange in biological soil crust communities under differential temperatures and soil water contents: implications for global change. Global Change Biology *, 16* (10), 2763-2774.

Hamerlynck, E., Csintalan, Z., Nagy, Z., Tuba, Z., Goodin, D., & Henebry, G. (2002). Ecophysiological consequences of contrasting microenvironments on the desiccation tolerant moss *Tortula ruralis*. Oecologia *, 131* (4), 498-505.

Hamerlynck, E., Tuba, Z., Csintalan, Z., Nagy, Z., Henebry, G., & Goodin, D. (2000). Diurnal variation in photochemical dynamics and surface reflectance of the desiccation-tolerant moss, *Tortula ruralis*. Plant Ecology , 151 (1), 55-63.

Hanson, D., Swanson, S., Graham, L., & Sharkey, T. (1999). Evolutionary significance of isoprene emission from mosses. American Journal of Botany 86(5): 634-639

Harper, K., & Pendleton, R. (1993). Cyanobacteria and cyanolichens: can they enhance availability of essential minerals for higher plants? Western North American Naturalist , 53 (1), 59-72.

Hinshiri, H., & Proctor, M. (1971). The effect of desiccation on subsequent assimilation and respiration of the bryophytes *Anomodon viticulosus* and *Porella platyphylla*. New Phytologist , 527-538.

Housman, D., Powers, H., Collins, A., & Belnap, J. (2006). Carbon and nitrogen fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert. Journal of arid environments , 66 (4), 620-634.

Huxman, T., Snyder, K., Tissue, D., Leffler, A., Ogle, K., Pockman, W., et al. (2004). Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. Oecologia , 141 (2), 1-15.

Jobson, R. W., & Qiu, Y.-L. (2010). Amino Acid Compositional Shifts During Streptophyte Transitions to Terrestrial Habitats. Journal of Molecular Evolution, 72(2), 204–214.

Kidron, G., Barzilay, E., & Sachs, E. (2000). Microclimate control upon sand microbiotic crusts, western Negev Desert, Israel. Geomorphology , 36 (1-2), 1-18.

Lackner, L. (1939) Uber die Jahresperiodizitat in der Entwicklung der Laub-moose. Planta 29: 534–616.

Larcher, W. 2003. Physiological Plant Ecology: Ecophysiology and stress physiology of functional groups. Springer, Berlin.

Li, Y., Wang, Z., Xu, T., Tu, W., Liu, C., Zhang, Y., & Yang, C. (2010). Reorganization of photosystem II is involved in the rapid photosynthetic recovery of desert moss *Syntrichia caninervis* upon rehydration. Journal of Plant Physiology, 167(16), 1390–1397.

Lioubimtseva, E. (2004). Climate change in arid environments: revisiting the past to understand the future. Progress in physical geography , 28 (4), 502.

Liu, Y., Cao, T., & Glime, J. (2003). The Changes of Membrane Permeability of Mosses under High Temperature Stress. The Bryologist , 106 (1), 53-60.

Loik, M., Breshears, D., Lauenroth, W., & Belnap, J. (2004). A multi-scale perspective of water pulses in dryland ecosystems: climatology and ecohydrology of the western USA. Oecologia , 141 (2), 269-281.

Long, S., Ainsworth, E., Rogers, A., & Ort, D. (2004). RISING ATMOSPHERIC CARBON DIOXIDE: Plants FACE the Future*. Annual Review of Plant Biology , 55 (1), 591-628.

Lovelock, C., Jackson, A., Melick, D., & Seppelt, R. (1995). Reversible photoinhibition in Antarctic moss during freezing and thawing. Plant physiology , 109 (3), 955.

Marschall, M. (2004). Are Bryophytes Shade Plants? Photosynthetic Light Responses and Proportions of Chlorophyll a, Chlorophyll b and Total Carotenoids. Annals of Botany , 94 (4), 593-603.

Martin, C.E. & Churchill S.P. 1982. Chlorophyll concentrations and a/b ratios in mosses collected from exposed and shaded habitats in Kansas. Journal of Bryology 12 (2): 297

Mcletchie, D. (2006). Sporophyte and Gametophyte Generations Differ in their Thermotolerance Response in the Moss Microbryum. Annals of Botany , 97 (4), 505-511.

Meehl, G.A., T.F. Stocker, W.D. Collins, P. Friedlingstein, A.T. Gaye, J.M. Gregory, A. Kitoh, R. Knutti, J.M. Murphy, A. Noda, S.C.B. Raper, I.G. Watterson, A.J. Weaver and Z.-C. Zhao (2007). Global Climate Projections. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC). Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Meyer, H., & Santarius, K. (1998). Short-term thermal acclimation and heat tolerance of gametophytes of mosses. Oecologia , 115 (1), 1-8.

Mishler, B., & Oliver, M. (1991). Gametophytic phenology of *Tortula ruralis*, a desiccation-tolerant moss, in the Organ Mountains of southern New Mexico. Bryologist , 143-153.

Mishler, B.D., and Oliver, M.J. (2009) Putting *Physcomitrella patens* on the tree of life: the evolution and ecology of mosses. Annual Plant Reviews, 36, 1-15

Mohamed, H., Thai, Y., & Gunaseelan, G. (2004). Additions to the moss flora of Peninsular Malaysia. Journal of Bryology , 26 (1), 47-52.

Ochyra, R., & Zander, R. (2002). The genera *Didymodon* and *Bryoerythrophyllum* (Pottiaceae) in Antarctica. Journal of Bryology , 24 (1), 33-44.

Oliver, M.J. & Bewley, J.D. (1984) Plant Desiccation and Protein Synthesis V. Stability of Poly(A)- and Poly(B)+ RNA during desiccation and their synthesis upon rehydration in the desiccation tolerance moss *Tortula ruralis* and the intolerant moss *Cratoneuron filicinum*. Plant Physiology, 74, 917-922

Oliver, M.J., Mishler, B.D., & Quidenberry, J.E. (1993). Comparative measures of desiccation-tolerance in the *Tortula ruralis* complex. I. Variation in damage control and repair. American Journal of Botany, 80(2), 127-136

Oliver, M.J., Velten, J., & Wood, A.J. (2000). Bryophytes as experimental models for the study of environmental stress tolerance: *Tortula ruralis* and desiccation tolerance in mosses. Plant Ecology, 151, 73-84

Oliver, M.J., & Tuba, Z., & Mishler, B.D. (2000). The evolution of vegetative desiccation tolerance in land plants. Plant Ecology, 151: 85-100

Oliver, M.J., & Velten, J., & Mishler, B.D. (2005). Desiccation tolerance in bryophytes: a reflection of the primitive strategy for plant survival in dehydrating habitats? Integrative and Comparative Biology, 45, 788-799

Paolillo, D. J., JR. (1979) On the lipids of the sperm masses of three mosses. The Bryologist 82: 93–96.

Proctor, M.C.F. 1979a Structure and eco-physiological adaptation in bryophytes. In Clarke, G.C.S. & Duckett, J.G. (eds), Bryophyte Systematics. Systematics association special volume No. 14 Academic press, London.

Proctor, M. (2003). Experiments on the effect of different intensities of desiccation on bryophyte survival, using chlorophyll fluorescence as an index of recovery. Journal of Bryology , 25 (3), 201-210.

Proctor, M. (2004). How long must a desiccation-tolerant moss tolerate desiccation? Some results of 2 years' data logging on *Grimmia pulvinata*. Physiologia Plantarum , 122 (1), 21-27.

Proctor, M. (2000). The bryophyte paradox: tolerance of desiccation, evasion of drought. Plant Ecology , 151 (1), 41-49.

Proctor, M., & Smirnoff, N. (2000). Rapid recovery of photosystems on rewetting desiccation-tolerant mosses: chlorophyll fluorescence and inhibitor experiments. Journal of Experimental Botany , 51 (351), 1695.

Proctor, M.C.F., & Pence, V.C. 2002. Vegetative tissues: bryophytes, vascular resurrection plants and vegetative propagules. In Desiccation and survival in plants: drying without dying. Black, M. & Pritchard, H.W., Eds.

Proctor, M.C.F. & Tuba, Z. (2002). Poikilohydry and homoihydry: antithesis or spectrum of possibilities? New Phytologist, 156, 327-349

Proctor, M., Nagy, Z., Csintalan, Z., & Takács, Z. (1998). Water-content components in bryophytes: analysis of pressure-volume relationships. Journal of Experimental Botany , 49 (328), 1845.

Proctor, M., Oliver, M., Wood, A., Alpert, P., Stark, L., Cleavitt, N., et al. (2007). Desiccation-tolerance in bryophytes: a review. The Bryologist , 110 (4), 595-621.

Proctor, M.C.F. & Smirnoff, N. (2011). Ecophysiology of photosynthesis in bryophytes: major roles for oxygen photoreduction and non-photochemical quenching? Physiologia Plantarum, 141, 130-140.

Proctor, M.C.F. 2008. Physiological Ecology. In Shaw, A.J. & Goffinet, B. (eds.) Bryophyte Biology. Cambridge University Press. Cambridge, U.K.

Reed, S.C., Coe, K.K., Sparks, J.P., Housman, D.C., Zelikova, T.J., & Belnap, J. (in review) Increased precipitation caused rapid moss mortality and altered fertility in a dryland ecosystem. (Nature Climate Change, in review)

Reynolds, J., Kemp, P., Ogle, K., & Fernandez, R. (2004). Modifying the pulse-reserve paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. Oecologia , 141 (2), 1-17.

Reynolds, R., Belnap, J., Reheis, M., Lamothe, P., & Luiszer, F. (2001). Aeolian dust in Colorado Plateau soils: nutrient inputs and recent change in source. Proceedings of the National Academy of Sciences , 98 (13), 7123.

Rosentreter, R., Bowker, M., and Belnap, J. 2007. A field guide to biological soil crusts of Western U.S. drylands. U.S. Government Printing Office, Denver, CO.

Sala, O., & Lauenroth, W. (1982). Small rainfall events: an ecological role in semiarid regions. Oecologia , 53 (3), 301-304.

Schlesinger, W., Raikes, J., Hartley, A., & Cross, A.F. (1996). On the spatial pattern of soil nutrients in desert ecosystems. Ecology, 77(2), 364-374

Schonbeck, M.W. & Bewley, J.D. 1981(a) Responses of the moss *Tortula ruralis* to desiccation treatments. I. Effects of minimum water content and rates of dehydration and rehydration. Canadian Journal of Botany, 59, 2698-2706.

Schonbeck, M.W. & Bewley, J.D. 1981(b) Responses of the moss *Tortula ruralis* to desiccation treatments. II. Variations in desiccation tolerance. Canadian Journal of Botany, 59, 2707-2712.

Schwinning, S., Starr, B., Wojcik, N., Miller, M., Ehleringer, J., & Sanford Jr, R. (2005). Effects of nitrogen deposition on an arid grassland in the Colorado plateau cold desert. Rangeland Ecology & Management , 58 (6), 565-574.

Seager, R., & Vecchi, G. (2010). Climate Change and Water in Southwestern North America Special Feature: Greenhouse warming and the 21st century hydroclimate of southwestern North America. Proceedings of the National Academy of Sciences , 107 (50), 21277-21282.

Seel, W.E., Hendry, G.A.F., and Lee, J.A. 1992. The Combined Effects of Desiccation and Irradiance on Mosses from Xeric and Hydric Habitats. J. Exp. Bot. 43 (8): 1023-1030

Sharkey, T.D. & Schrader, S.M. (2006) High Temperature Stress. in Madhava Rao, K.V., Raghavendra, A.S., & Reddy K.J. (Eds) Physiology and Molecular Biology of Stress Tolerance in Plants. Springer. Dordrecht, The Netherlands. pp101-130

Sharkey, T.D., & Yeh, S. (2001). Isoprene Emission from Plants. Annual Reviews of Plant Physiology and Plant Molecular Biology, 52, 407-436

Sharkey, T.D., Badger, M.R., von Caemmerer, S., & Andrews, T. J. (2001). Increased heat sensitivity of photosynthesis in tobacco plants with reduced Rubisco activase. Photosynthesis Research, 67, 147-156

Smirnoff, N. 1992. The carbohydrates of bryophytes in relation to desiccation tolerance. Journal of Bryology (17) 185-198.

Stark, L.R., Mishler, B.D., & Mcletchie, D. N. (2000). The cost of realized sexual reproduction: assessing patterns of reproductive allocation and sporophyte abortion in a desert moss. American Journal of Botany 87(11), 1599-1608

Stark, L. R. (2001). Widespread Sporophyte Abortion Following Summer Rains in Mojave Desert Populations of Grimmia orbicularis. The Bryologist, 104(1), 115–125

Stark, L. (2005). Phenology of patch hydration, patch temperature and sexual reproductive output over a four-year period in the desert moss *Crossidium crassinerve*. Journal of Bryology , 27 (3), 231-240.

Stark, L., Brinda, J. C., & McLetchie, D.N. (2011). Effects of increased summer precipitation and N deposition on Mojave Desert populations of the biological crust moss *Syntrichia caninervis.* Journal of Arid Environments, 75, 457-463

Stark, L., & McLetchie, D. (2006). Gender-specific heat-shock tolerance of hydrated leaves in the desert moss *Syntrichia caninervis*. Physiologia Plantarum , 126 (2), 187-195.

Stark, L., Mcletchie, D., & Eppley, S. (2010). Sex ratios and the shy male hypothesis in the moss *Bryum argenteum* (Bryaceae). The Bryologist , 113 (4), 788-797.

Stark, L., McLetchie, D., & Mishler, B. (2005). Sex expression, plant size, and spatial segregation of the sexes across a stress gradient in the desert moss *Syntrichia caninervis*. The Bryologist , 108 (2), 183-193.

Stark, L.R., Oliver, M.J., Mishler, B.D., & McLetchie, D.N. (2007). Generational differences in response to desiccation stress in the desert moss *Tortula inermis*. Annals of botany, 99, 53-60

Stark, L.R., McLetchie, D.N., Smith, S.D., and Oliver, M.J. 2011. Responses of a biological soil crust moss to increased monsoon precipitation and nitrogen deposition in the Mojave desert. In Tuba, Z., Slack, N. & Stark L.R. (eds.) Bryophyte Ecology and Climate Change. Cambridge Univ. Press.

Thompson, D., Walker, L., Landau, F., & Stark, L. (2005). The influence of elevation, shrub species, and biological soil crust on fertile islands in the Mojave Desert, USA. Journal of Arid Environments , 61 (4), 609-629.

Tuba, Z., Csintalan, Z., & Proctor, M. (1996). Photosynthetic responses of a moss, *Tortula ruralis, ssp. ruralis*, and the lichens *Cladonia convoluta* and *C. furcata* to water deficit and short periods of desiccation, and their ecophysiological significance: a baseline study at present-day CO_2 concentration. New Phytologist , 353-361.

Tuba, Z., Csintalan, Z., Szente, K., Nagy, Z., & Grace, J. (1998). Carbon gains by desiccation-tolerant plants at elevated CO2. Functional Ecology , 12 (1), 39-44.

Tuba, Z., Lichtenthaler, H., Csintalan, Z., Nagy, Z., & Szente, K. (1994). Reconstitution of chlorophylls and photosynthetic CO_2 assimilation upon rehydration of the desiccated poikilochlorophyllous plant *Xerophyta scabrida* (Pax) Th. Dur. et Schinz. Planta , 192 (3), 414-420.

Tuba, Z., & Lichtenthaler, H.K. (2011). Ecophysiology of Homoiochlorophyllous and Poikilochlorophyllous Desiccation-Tolerant Plants and Vegetations. In U. Luttge et al. (eds)

Plant Desiccation Tolerance. Ecological Studies 215, Springer-Verlag Berlin Heidelberg, Germany

Tucker, E., Costerton, J., & Bewley, J. (1975). The ultrastructure of the moss *Tortula ruralis* on recovery from desiccation. Canadian Journal of Botany , 53 (2), 94-101.

Valko, P.G. 2003. Monitoring Biological Soil Crusts using Hyperspectral Remote Sensing: Determination of Cyanobacteria, Lichen, and Moss Contribution to Spectral Indices and Observing Community Compositional Changes due to Global Climate Change. Senior Thesis, George Washington University, St. Louis.

Valanne, N. 1984. Photosynthesis and Photosynthetic Products in Mosses. In Dyer, A. J. and Duckett ,J.G. (eds.) The Experimental Biology of Bryophytes: 257-273

Van Auken, O. (2000). Shrub invasions of North American semiarid grasslands. Annual Review of Ecology and Systematics, 31, 197-215.

Vitousek, P., & Howarth, R. (1991). Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry , 13 (2), 87-115.

Wal, R., Pearce, I., & Brooker, R. (2005). Mosses and the struggle for light in a nitrogen-polluted world. Oecologia , 142 (2), 159-168.

Walker, L.R.,Thompson, D. B., & Landau, F.H. (2001). Experimental manipulations of fertile islands and nurse plant effects in the Mojave Desert, USA. Western North American Naturalist, 61(1), 25-35

Williams, T., & Flanagan, L. (1998). Measuring and modelling environmental influences on photosynthetic gas exchange in Sphagnum and Pleurozium. Plant, Cell & Environment , 21 (6), 555-564.

Xu, S., Jiang, P., Wang, Z., & Wang, Y. (2009). Crystal structures and chemical composition of leaf surface wax depositions on the desert moss *Syntrichia caninervis*. Biochemical Systematics and Ecology, 37, 723-730

Xu, S.-j., Liu, C.-j., Jiang, P.-a., Cai, W.-m., & Wang, Y. (2009). The effects of drying following heat shock exposure of the desert moss *Syntrichia caninervis*. Science of the Total Environment, The , 407 (7), 2411-2419.

Zaady, E., Kuhn, U., Wilske, B., Sandoval-Soto, L., & Kesselmeier, J. (2000). Patterns of CO_2 exchange in biological soil crusts of successional age. Soil Biology and Biochemistry , 32 (7), 959-966.

CHAPTER 2

Physiological ecology of desert biocrust moss following 10 years exposure to elevated CO_2: evidence for enhanced photosynthetic thermotolerance[2]

[2] Originally published as **Coe, K.K**., Belnap, J., Grote, E. & Sparks, J. (2012) Physiological ecology of the desert moss *Syntrichia caninervis* after ten years exposure to elevated CO_2: evidence for enhanced photosynthetic thermotolerance. *Physiologia Plantarum* 144(4), 346-356

Abstract

In arid regions, biomes particularly responsive to climate change, mosses play an important biogeochemical role as key components of biocrusts. Using the biocrust moss *Syntrichia caninervis* collected from the Nevada Desert Free Air CO_2 Enrichment Facility, we examined the physiological effects of 10 years of exposure to elevated CO_2, and the effect of high temperature events on the photosynthetic performance of moss grown in CO_2-enriched air. Moss exposed to elevated CO_2 exhibited a 46% decrease in chlorophyll, a 20% increase in carbon, no difference in either nitrogen content or photosynthetic performance, and no evidence for photosynthetic acclimation. However, when subjected to high temperatures (35–40°C), mosses from the elevated CO_2 environment showed higher photosynthetic performance and photosystem II (PSII) efficiency compared to those grown in ambient conditions, potentially reflective of a shift in nitrogen allocation to components that offer a higher resistance of PSII to heat stress. This result suggests that mosses may respond to climate change in markedly different ways than vascular plants, and observed CO_2-induced photosynthetic thermotolerance in *S. caninervis* will likely have consequences for future desert biogeochemistry.

Introduction

Non-vascular plants (mosses and their relatives) are an important component of global net primary productivity (Campioli et al. 2009, Lindo and Gonzalez 2010), biogeochemistry (Turetsky 2003) and biodiversity (Molau and Alatalo 1998, Shaw et al. 2005). Because they are photosynthetically similar to their vascular counterparts, it is often assumed that these plants will

likely be similarly responsive to elevated atmospheric CO_2. However, mosses do not possess the same regulatory mechanisms associated with gas exchange and sugar transport that define vascular plant responses to long-term CO_2 fumigation, so their response to a future atmosphere is likely to be different.

In vascular plants grown in elevated CO_2, the increased substrate for photosynthesis has a threefold impact on physiological ecology: photorespiration is reduced and carboxylation efficiency increases (Sage et al. 1989, Bazzaz 1996, Cousins et al. 2001, Long et al. 2004), stomatal aperture is reduced and as a consequence plant water use efficiency increases (Bazzaz 1990, Long et al. 2004, Ainsworth and Rogers 2007) and sucrose production is enhanced in leaves (Long et al. 2004). Therefore, vascular plant response to short-term (1–3 years) enrichment includes stimulation of photosynthesis, productivity and biomass accumulation (Norby et al. 1999, Nowak et al. 2004, Ainsworth and Long 2005, Saxe et al. 2008). In the long term (>8 years), however, initial increases in production are usually offset by the acclimation of photosynthetic rates to align with source-sink carbon (C) relations (downstream limitation in phloem-linked networks) and nutrient availability (Sage et al. 1989, Long et al. 2004). Because of the reduced demand for cellular mechanisms involved in C capture and processing under long-term CO_2 enrichment, this response also usually leads to a decrease in the function and amount of Rubisco as well as an overall decrease in nitrogen (Long et al. 2004, Ainsworth and Long 2005, Leakey et al. 2009).

Most moss gametophytes, possessing neither stomata nor complex vascular tissue, are likely to respond to elevated CO_2 differently. First, gas exchange is not actively regulated via the stomatal aperture and instead CO_2 concentration within chloroplasts (c_c) depends on diffusion of air through the boundary layer, external capillary water, water-saturated cell walls and plasma

membranes to points of carboxylation. Similar to vascular plants, c_c in mosses is heavily influenced by photosynthetic rate, but because water limits the diffusion of CO_2, the primary environmental controls on c_c in mosses tend to be shoot water content (Williams and Flannagan 1998) that varies with microclimate water availability. Second, mosses lack phloem, and although transport distances are short and cellular export of sugars is likely (Ligrone et al. 2000), mosses possess neither the extensive transport mechanisms necessary to translocate sugars over long distances nor the ability to regulate carbohydrate production based on sink strength or phloem-loading characteristics. Therefore, response to prolonged elevated CO_2 cannot include active suppression of the Calvin cycle or differences in water use in a manner analogous to vascular plants.

To date, moss responses to CO_2 enrichment have been measured in several peatland species, where biomass production has been observed to either be increased (Tuba et al. 1998, Heijmans et al. 2001) or not influenced (Berendse et al. 2001, Toet et al. 2006) after 1–3 years of treatment, and in one dryland species (Brinda et al. 2011) where sexual reproduction was enhanced and improved desiccation tolerance was observed. Soluble sugars in *Sphagnum* spp. have been shown to increase by 20–30% after short-term CO_2 exposure, and nitrogen (N) content either declines or remains constant (Tuba et al. 1998, Berendse et al. 2001, Heijmans et al. 2001). With the exception of recent work by Brinda et al. (2011), no work to date has examined responses beyond three years of elevated CO_2 treatment, and we currently possess very limited knowledge on responses of non-mesic mosses.

In arid and semi-arid systems, mosses often play a disproportionately large ecological role due to their presence in biocrust communities (composed of mosses, lichens and cyanobacteria) that are essential in the biogeochemical cycling of nutrients in these regions.

Crusts can account for up to 70% of ground cover in deserts of the Southwestern United States where they fix substantial amount of C and N (Evans and Lange 2003, Housman et al. 2006), can act as the primary source of N to plant and soil communities (Evans and Ehleringer 1993), aid in seedling establishment (Su et al. 2007), increase water retention of soil, and decrease erosion (Belnap and Gillette 1998, Belnap 2003). Mosses within biocrusts appear to be particularly sensitive to climate change, and declines in moss have been correlated with reduced overall desert biocrust functionality (Belnap, unpublished data).

Deserts of the Southwestern United States, in addition to experiencing the global 250–500 ppb increase in CO_2 within the next century (Meehl et al. 2007), are predicted to be subjected to dramatic (4–6°C) average temperature increases as well as more frequent extreme high temperature events (Bazzaz 1996, Wagner 1996, Meehl et al. 2007). As these regions are likely to simultaneously experience elevated CO_2 and high temperatures in the foreseeable future (Meehl et al. 2007), it is likely that these factors will act in concert to impact plant physiology and functional ecology. Understanding how desert plants, particularly biocrust mosses, growing in an enriched atmosphere will respond to high temperature events will therefore be instrumental in predictions for arid ecosystem function.

Some recent work has suggested that short-term exposure to elevated CO_2 enhances temperature tolerance of photosynthesis in plants (Faria et al. 1996, Huxman et al. 1998, Hamerlynck et al. 2000a, 2000b, Taub et al. 2000, Wang et al. 2008). Based on the climate change scenarios, such thermotolerance mechanisms may be particularly important for desert plants. The ability to withstand high temperatures during physiological functioning is already evolutionarily adaptive and present in this biome (Berry and Bjorkman 1980, Downton et al. 1984, Loik and Harte 1996); therefore, mechanisms involved in photosynthetic thermotolerance

are most likely a component of general function (Knight and Ackerly 2002, 2003). Desert mosses are a likely candidate for possession of elevated CO_2-induced thermotolerance of photosynthesis, with clear consequences for biocrust functioning in this region, yet the presence of such mechanisms have to be identified in this plant functional group.

In this study, we examined the effects of long-term (10 years) elevated CO_2 exposure on the common desert moss *Syntrichia caninervis* (Mitten) collected from the Nevada Free Air CO_2 Enrichment (FACE) Facility in the Mojave Desert, and were interested in the physiological and photosynthetic responses to long-term CO_2 enrichment as well as the temperature tolerance of photosynthesis for plants grown in CO_2 enriched air. Specifically, we predicted that (1) desert moss exposed to 10 years of CO_2 enrichment will exhibit the stimulatory effects of increased C substrate (including higher photosynthetic rates and increased C uptake), yet will not downregulate biochemical fixation ability in the long-term because the limitations imposed by stomata and phloem transport are not present, and (2) desert moss, already possessing thermotolerance mechanisms due to their growth environment, will exhibit enhanced photosynthetic thermotolerance as a consequence of 10 years of elevated CO_2 exposure.

Methods

Growth conditions and field collection

Samples of *S. caninervis* were collected from the Nevada Desert FACE Facility (NDFF), located approximately 80 km north of Las Vegas, NV, USA. Constructed in 1997, the NDFF was designed to measure the effects of CO_2 enrichment on an intact Mojave Desert ecosystem. The NDFF is comprised of nine, 25-m diameter rings that enclose naturally occurring Mojave Desert

vegetation. Using attached blowers, each plot is continuously fumigated from the surrounding ring with either ambient air (approximately 360 ppm CO_2) or CO_2-enriched air (550 ppm CO_2; n = 3 rings per treatment), and an additional three rings were left unfumigated as a control. At the time of collection, moss had been subjected to treatments for 10 years (April 1997–April 2007) during which time the environmental conditions at the NDFF included annual temperatures between −10 and 45°C, and mean annual precipitation of approximately 140 mm. Additional details of the NDFF design and site characteristics are described in Jordan et al. (1999).

Samples of *S. caninervis* were collected on April 17 and 18, 2007, from 360 ppm control and 550 ppm treatment plots just after CO_2 treatments ceased at the NDFF. Moss samples (n = 72), and the underlying organic layer (<1 cm below the soil surface), were carefully lifted off the ground using a dissecting probe and placed in labeled herbarium packets documenting the ring number, latitude and longitude. Samples were collected from rings wherever patches were large enough (>5 cm^2) and the number of samples collected in each ring differed slightly due to heterogeneity in moss distribution. Following collection, moss samples were immediately transported to the USGS Canyonlands Research Station in Moab, UT, USA, for analyses. All samples were collected and transported in the desiccated state to eliminate exposure to (and reaction with) ambient atmospheric conditions prior to experimentation.

Sample pre-treatment

A 2-cm diameter section of each sample was thoroughly rinsed with distilled water to remove soil and placed in a numbered gold-plated copper cap lined with filter paper. Each copper cap had small holes to allow drainage of excess water. Following rinsing and drainage, each sample was assumed to be at 100% relative water content (RWC). Groups of four samples

were placed in Petri dishes and incubated at 30°C for approximately 90 min prior to measurement. This controlled incubation served to (1) ensure sufficient rehydration time for commencement of physiological activity and (2) attain a standard RWC for photosynthesis measurements (which was approximately 50%; data not shown). These conditions are not typical for bryophyte pre-treatment (where samples are allowed to dry more slowly) and for this study represented a trade-off between drying samples to a level where optimum photosynthetic rates were reached, yet simultaneously minimizing exposure to ambient air. Due to the small amount of available plant material, we used samples primarily for gas exchange (18 samples consisting of four caps each) and chlorophyll fluorescence (30 caps analyzed prior to gas exchange). Elemental analysis and pigment information were gathered on samples following gas exchange measurements.

Photosynthetic gas exchange

We used laboratory-based CO_2 exchange analysis to examine the effect of 10 years of elevated CO_2 on photosynthetic performance of *S. caninervis* and to determine the effect of high temperatures on photosynthetic functioning. Traditionally, A–ci analyses, the leaf photosynthetic rate measured over a range of leaf-internal CO_2 concentrations, are used to examine diffusional and biochemical limitations to photosynthesis in higher plants (Farquhar et al. 1980). However, because mosses possess a different set of resistances to CO_2 diffusion compared to vascular plants, an A–c_i curve cannot be resolved to generate estimates of Vcmax (limitation to photosynthesis due to CO_2 diffusion) and J_{max} (limitation due to electron transport). First, c_i (thus c_c) cannot be estimated in bryophytes simply from gas exchange and has been shown to vary primarily as a function of shoot water content (Williams and Flannagan 1998). Therefore, all

measurements presented in this study were made at a constant shoot water content. Additionally, after passing through the boundary layer, the resistance path of CO_2 into chloroplasts of mosses is exclusively a mesophyllic resistance because the diffusion path does not include stomata. Internal CO_2 concentration in vascular plants is often defined as the CO_2 concentration at sites of evaporation (i.e. the substomatal cavity surface) rather than the point of carboxylation; yet in mosses, the analogous evaporative surface is the entire gametophyte that is exposed to CO_2 in the surrounding air. Finally, boundary layer conductance to CO_2 was assumed to be large in this experiment (based on the construction of our cuvette and the uniform depth of green tissue in tightly packed moss colonies), therefore external CO_2 concentration (c_a) can be used to approximate CO_2 at the leaf surface (c_s). Thus, while we could not use the standard A–c_i technique in this study, we performed a similar analysis by measuring CO_2 exchange (A) as a function of external CO_2 concentration (c_a) under constant light and humidity.

Since it is of the same supply–demand form as an A–c_i curve, a photosynthesis-response curve for mosses at a constant water content created from an analysis of A as a function of c_a produces two parameters that can be compared across treatments: initial slope (m_i; indicative of the limitation to photosynthesis imposed by the diffusional resistance imposed by the CO_2 pathway from the atmosphere to the site of carboxylation) and maximum rate (f_{max}; maximum CO_2 flux, indicative of the limitation imposed by inherent biochemistry at maximum CO_2 consumption – either as the rate of regeneration of RUBP through the Calvin cycle or as the activity of Rubisco). In this study, differences in the parameters, m_i and f_{max}, across CO_2 and/or temperature levels thus reflect differences in photosynthetic performance imposed by treatments.

For gas exchange analysis, groups of four moss samples were placed in a nickel-plated, custom-built cuvette attached to a desktop gas exchange system. The gas exchange system

consisted of a gas mixer (Environics Series 4000; Environics Inc., Tolland, CT), humidifier, custom-built cuvette and an infrared gas analyzer (Li-6262; Li-COR Inc., Lincoln, NE) and was run with the program LabView (National Instruments Corporation, Houston, TX). Internal conditions of the cuvette were maintained at approximately 800 μmol m^{-2} s^{-1} photosynthetically active radiation and approximately 65% relative humidity. The cuvette was temperature controlled (±0.5°C) using a water bath (Fisher Isotemp 105; Thermo Fisher Scientific Inc., Waltham, MA) and a coil system built into the cuvette. We used thermocouples to continuously monitor air and moss temperature within the cuvette over the course of the experiment (the difference between air and moss temperatures never varied >1°C).

We measured maximum CO_2 assimilation rates for *S. caninervis* grown under ambient and elevated CO_2 treatments at the following sequence of CO_2 concentrations (c_a; ppm): 375, 250, 150, 50, 150, 250, 375, 500, 800 and 1000, where c_a is the CO_2 level exiting the chamber. Each group of four samples was allowed 5 min to acclimate to each concentration prior to measurements at low CO_2 levels (<375 ppm) and 10–15 min to acclimate to higher concentrations (times previously determined by the stabilization of CO_2 levels in the chamber). Gross photosynthetic rate (A_{gross}) was calculated as the sum of maximum CO_2 assimilation (A_{net}) in light conditions and dark respiration values at each CO_2 level. Respiration rates did not differ between samples grown in enriched and ambient conditions; therefore, A_{gross} is proportionally equivalent to A_{net} for all measurements. Samples were kept at an RWC approximately 50% by weight (distilled water was added with a dropper to individual samples as needed) throughout all gas exchange measurements. Moss photosynthetic rate (A) was plotted as a function of CO_2 concentration (c_a) to create response curves for moss samples from each treatment, and m_i and f_{max} were estimated from each curve using curve fitting.

Gas exchange measures were conducted at three leaf temperatures: 20, 30 and 40°C, with the 40°C level designed to simulate temperatures that fall within the range of predicted values under future climate scenarios. Soil temperatures in the Mojave Desert often exceed 40°C in the summer. However, currently mosses in this system are inactive and desiccated during this time of the year. If summer rainfall increases in the future as predicted by some modeling efforts, then these temperatures would be experienced by mosses in a hydrated state. We chose 40°C as our maximum temperature treatment because (1) it falls just below a heat shock threshold at which photochemical destruction (Xu et al. 2009) and declines in reproductive success and growth (Stark and McLetchie 2006) occur in *S. caninervis* and (2) it represents a relevant temperature stress level under which mosses are likely to be hydrated and physiologically active under future climate scenarios.

Chlorophyll fluorescence

We used the chlorophyll fluorescence parameter, F_v/F_m, to examine differences in photosynthetic efficiency in *S. caninervis* from elevated and ambient CO_2 treatments. Following incubation procedures described above, 30 moss samples were dark-adapted for 15 min [previously shown to be sufficient to dissipate non photochemical quenching (NPQ)] and pulse amplitude-modulated (PAM) fluorescence was measured using a PAM-2000 fluorometer (H. Walz, Effeltrich, Germany). Baseline fluorescence (F_o) of samples exposed to an actinic light was measured prior to exposure to saturating pulses. Using a spacer to ensure constant sample distance of 1 cm from the light source, maximum fluorescence (F_m) was driven by a 0.8-s saturating pulse (3000 mol m^{-2} s^{-1} PPFD) propagated through a fiber optic cable. Maximum

photochemical efficiency of photosystem II (PSII) was calculated as $F_v/F_m = (F_m - F_o)/F_m$ (Shreiber 1998).

Tissue analysis

To assess changes in relative allocation of N to light capture for photosynthesis across treatments (a measure of acclimation to elevated CO_2), we measured pigmentation levels in moss from elevated and ambient CO_2 plots. Chlorophyll a and b, β-carotene, violaxanthin and lutein content of *S. caninervis* were quantified using high performance liquid chromatography (HPLC), as described by Garcia-Pichel and Castenholz (1991). Moss samples (n = 58) were removed from herbarium packets, thoroughly rinsed of soil and debris, left to dry overnight in the light, then mixed well using a mortar and pestle and weighed to the nearest 0.001 g. Each sample was ground using a mixer mill (Spex Certi-prep Inc., Metuchen, NJ) for 10 min at 27 Hz, then 1/2 ml of distilled water was added to the sample, followed by 10 ml of acetone. Solutions were vortexed daily and allowed to sit for two nights, filtered with Whatman GF/F glass-fiber filter paper, then evaporated down to 3 ml using a warm water bath and a mild stream of N prior to HPLC analysis. Concentrations for all pigments were quantified using peak areas integrated from a photodiode array data at 436 nm (Garcia-Pichel et al. 1992, Belnap et al. 2004). Results were expressed as mg pigment g^{-1} moss tissue.

To determine the effect of long-term CO_2 enrichment on C and N levels in moss shoots, elemental analysis of *S. caninervis* tissue was conducted at the Cornell University Stable Isotope Laboratory. To prepare tissue for analysis, moss gametophytes were immersed in water and green portions were separated from brown (below-ground) sections. Green tissue samples were then pulverized with a spatula, weighed out to approximately 2.5 mg and then stored in a

desiccant jar prior to analysis. Samples (n = 20) were loaded into an NC2500 Carlo Erba elemental analyzer coupled to a Thermo Delta Plus isotope ratio mass spectrometer (Thermo Fisher Scientific Inc., Thermo Electron, Waltham, MA, USA), combusted at 1000°C and then passed through a chromium oxide column. Released NO_x gases were reduced to N_2 using a copper column at 650°C, and the resultant CO_2 and N_2 were passed through a magnesium perchlorate trap and then separated on a GC column. Percent N and C values were derived from sample peaks (including peak area, amplitude and sample weight).

Statistical analysis

One-way ANOVA (JMP version 7.0, SAS Institute Inc., Cary, NC) was used to determine differences between percent C and N of S. *caninervis* grown in ambient (360 ppm) and elevated (550 ppm) CO_2 environments. These data were arcsine transformed prior to statistical analysis to meet ANOVA distribution assumptions (Zar 1999). Student's t-tests were used to determine differences in mean F_v/F_m and in pigment concentration of samples grown in each of the CO_2 environments.

A two-way ANOVA was performed to analyze differences in m_i and f_{max} of samples from both CO_2 treatments under three different temperatures (20, 30 and 40°C) using CO_2 level and temperature as main factors in the model. Results are presented for the individual effects of temperature and CO_2 level on m_i and f_{max} as well as on the interactions between these factors. In addition, because we were specifically interested in the influence of CO_2 level on photosynthetic performance at high temperatures, we used a t-test assuming equal variance to analyze differences in m_i and f_{max} occurring during the simulated high-heat event (40°C). Results for

statistical analyses are shown for 95% confidence intervals and an $\alpha = 0.05$, unless otherwise noted.

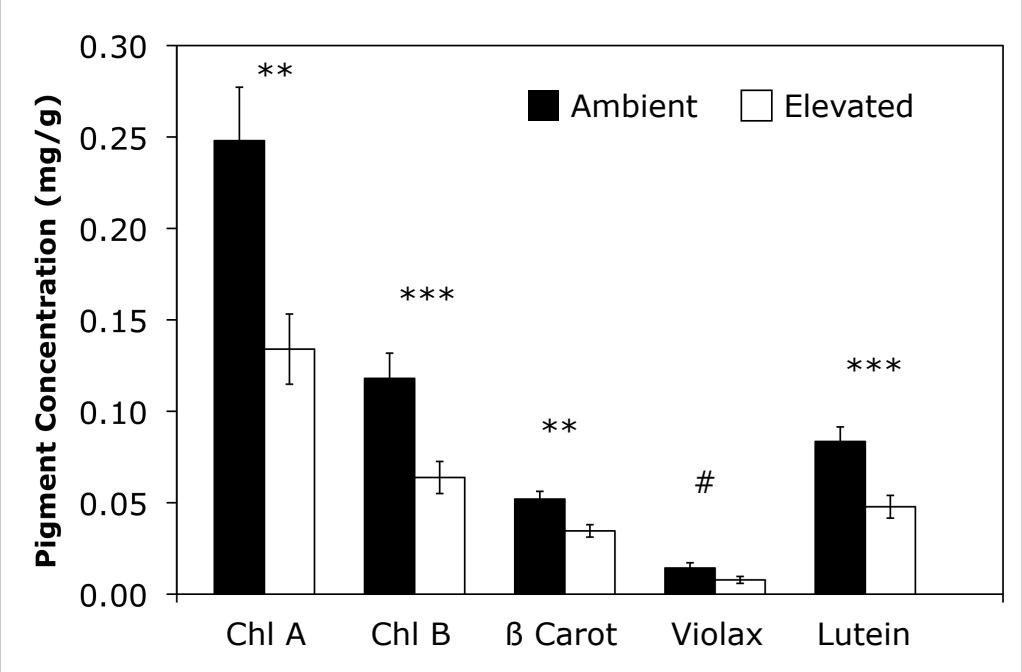


Figure 2.1: Pigment concentrations (chlorophyll *a*, chlorophyll *b*, *β*-carotene, violaxanthin and lutein; mg g^{-1}) of *Syntrichia caninervis* following 10 years exposure to ambient (360 ppm) or elevated (550 ppm) CO_2 (n = 58). Error = ±1 se; $^{**}P < 0.01$; $^{***}P < 0.001$; #$P < 0.1$.

Results

Mosses exposed to elevated CO_2 exhibited lower photosynthetic pigment levels when compared to samples grown under ambient conditions (Fig. 2.1). Chlorophyll levels decreased by approximately 46%; chlorophyll a decreased from 0.248 to 0.134 mg g^{-1} ($P < 0.01$) and chlorophyll b was reduced to 0.0638 from 0.118 mg g^{-1} ($P < 0.001$). β-Carotene levels were reduced from 0.052 to 0.035 mg g^{-1} ($P < 0.01$) and lutein levels decreased from 0.084 to 0.048 mg g^{-1} ($P < 0.001$). The concentration of violaxanthin in tissue decreased from 0.014 to 0.008 mg g^{-1} in samples from the elevated CO_2 treatment, but this difference was not significant at an

α = 0.05 ($P = 0.06$). When compared to individuals grown under ambient conditions, *S. caninervis* from the elevated CO_2 treatment exhibited a 20% increase in carbon (C) content ($F_{1,17} = 6.36$, $P < 0.05$) and no change in N content ($F_{1,17} = 0.10$, $P = 0.89$; Fig. 2.2). This resulted in an increase in C:N from 15.2 in mosses exposed to ambient conditions to 17.7 in those exposed to elevated CO_2 ($F_{1,17} = 7.13$, $P < 0.05$).

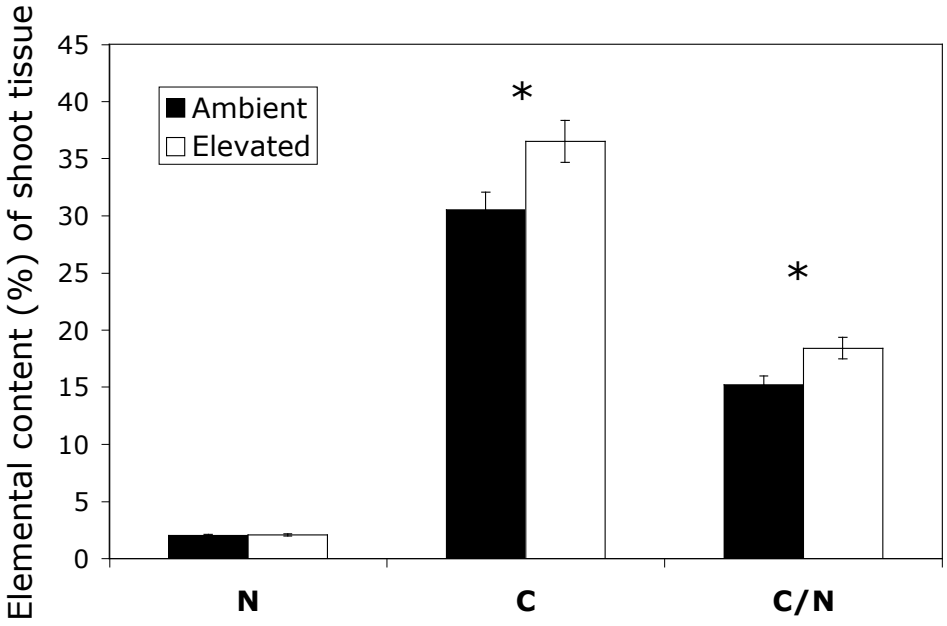


Figure 2.2: Mean carbon (C) and nitrogen (N) content (±1 se) of *Syntrichia caninervis* shoots from ambient (360 ppm) and elevated (550 ppm) CO_2 plots (n = 20). A one-way ANOVA was used to determine differences between CO_2 treatments (asterisks indicate differences where $P < 0.05$). Values for percent tissue composition were arcsine transformed prior to statistical analysis to meet anova distribution assumptions.

Temperature had a significant overall effect on carboxylation capacity (mi) and maximum rate of CO_2 flux (f_{max}) in *S. caninervis* (Table 2.2A). Moss measured at temperatures common during the growing season (20–30°C) displayed no difference in m_i or f_{max} for the two CO_2 environments (although values for both photosynthetic parameters were slightly higher at

30°C; Fig. 2.3, Table 2.1), thus the overall effect was driven by differences during the high temperature event. At 40°C, gross CO_2 assimilation rates were greatly reduced relative to the lower temperatures, with maximum values of approximately 3.5 μmol m^{-2} s^{-1} at 40°C compared to 8–10 μmol m^{-2} s^{-1} at 20–30°C (m_i: $F_{3,14} = 18.01$, $P < 0.0001$; f_{max}: $F_{3,14} = 15.03$, $P < 0.0001$). Photosynthetic performance (m_i and f_{max}) was also significantly reduced to <50% of levels from 20 to 30°C measurement temperatures during the high-heat event ($P < 0.05$; Table 2.1).

Table 2.1: Photosynthetic parameters estimated from CO_2-response curves, m_i (initial slope of curve; photosynthetic limitation due to CO_2 availability) and f_{max} (final slope of curve; photosynthetic limitation due to biochemistry at maximum C fixation rate) conducted at three temperatures for *Syntrichia caninervis* grown under ambient (360 ppm) and elevated (550 ppm) CO_2 conditions (n = 3 per treatment combination). Asterisks indicate significant difference from ambient controls ($P < 0.05$).

	m_i (μmol s^{-1} ppm CO_2^{-1})		**f_{max} (μmol m^{-2} s^{-1})**	
CO_2 treatment (ppm)	**360**	**550**	**360 ppm**	**550 ppm**
20 °C	37.2	37.1	10.4	9.94
30 °C	48.5	45.9	12.7	12.5
40 °C	7.49	17.5*	2.46	4.77*

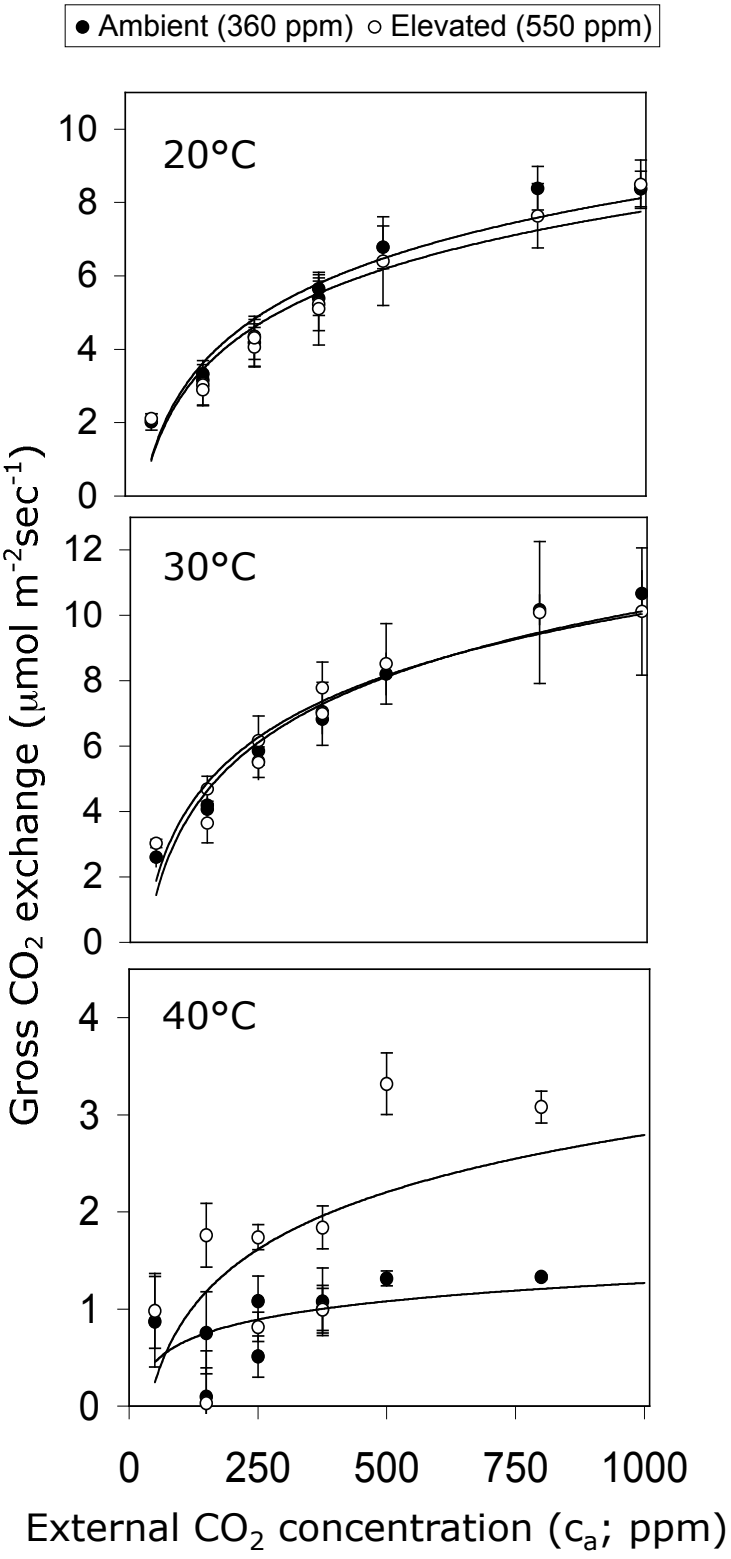
The overall effect of CO_2 level on photosynthetic performance (m_i and f_{max}) was not significant over the entire range of temperatures nor was the interaction with temperature (Table 2.2A) because m_i and f_{max} were not influenced by CO_2 at the 20–30°C level (Fig. 2.3, Table 2.1). However, analysis of these parameters during only the 40°C simulated event showed that CO_2 treatment strongly influenced photosynthetic performance under high temperature conditions (Fig. 2.3, Table 2.2B). Assimilation rates of moss from the elevated CO_2 treatment were not reduced to the extent of those grown at ambient levels (i.e. they displayed higher photosynthetic performance) and consequently exhibited m_i and f_{max} values double that of moss grown under

ambient CO_2 conditions ($F_{1,4} = 20.02$, $P = 0.011$ and $F_{1,4} = 9.62$, $P = 0.036$, respectively). Further, moss grown in elevated (550 ppm) CO_2 also exhibited a 15% increase in the efficiency of PSII when measured at 35°C ($P < 0.05$), shown by an average F_v/F_m value of 0.578 (±0.01) compared to a value of 0.512 (±0.02) for moss from ambient (360 ppm) conditions.

Table 2.2: (A) Results from two-way ANOVA on the singular and interactive effects of CO_2 level (360 and 550 ppm) and temperature (20, 30 and 40°C) on the photosynthetic parameters, m_i and f_{max}, for *Syntrichia caninervis*. Asterisks indicate significant effects ($\alpha = 0.05$) of treatments on m_i and f_{max}. (B) Analysis of the effect of CO_2 level on m_i and f_{max} of *S. caninervis* during the simulated high-heat event (40°C) using an equal variance *t*-test. Asterisks indicate significant differences ($\alpha = 0.05$) in either m_i or f_{max} as a function of CO_2 treatment.

A				
Source	**d.f.**	**Sum of squares**	**F**	***P***
m_i				
CO_2	1	0.576	0.1148	0.7406
Temperature	2	249.96	24.915	<0.0001*
CO_2× temperature	2	4.413	0.4399	0.6541
f_{max}				
CO_2	1	19.261	0.227	0.6427
Temperature	2	3634.9	21.37	0.0001*
CO_2× temperature	2	114.02	0.67	0.5297
B 40°C				
m_i				
CO_2	1	4.593	9.62	0.0362*
Error	4	1.909	–	–
Total	5	6.503	–	–
f_{max}				
CO_2	1	122.58	20.015	0.0110*
Error	4	24.497	–	–
Total	5	147.08	–	–

Figure 2.3: Gross CO_2 assimilation (μmol m^{-2} s^{-1}; ±1 se) as a function of cuvette air CO_2 concentration (c_a) for *Syntrichia caninervis* grown at ambient (360 ppm, filled symbols) and elevated (550 ppm, open symbols) CO_2 at 20, 30, and 40°C (symbols represent mean values from 3 replicates; 18 replicates total). Due to instrument limitations, data are not presented for the 1000 ppm CO_2 level at 40°C because this concentration and temperature combination could not be maintained in the chamber.


● Ambient (360 ppm) ○ Elevated (550 ppm)
20°C
30°C
40°C
Gross CO_2 exchange (μmol $m^{-2}sec^{-1}$)
External CO_2 concentration (c_a; ppm)
0
250
500
750
1000

Discussion

Exposure of the desert moss *S. caninervis* to 10 years of CO_2 enrichment altered the tissue composition and physiology of this species, both in terms of the form and function of the photosynthetic apparatus and with respect to tissue C:N.

We predicted that 10 years of elevated CO_2 treatment would increase C acquisition in *S. caninervis*, and we did observe increased C content (Fig. 2.2), but the photosynthetic performance of moss from elevated CO_2 plots was not different from moss grown under ambient conditions when measured at temperatures between 20 and 30°C (Fig. 2.3, Table 2.1). Long-term elevated CO_2 did not alter parameters associated with the supply of CO_2 or rates of carboxylation (m_i and f_{max}) suggesting that this moss did not downregulate biochemical fixation ability in response to treatment. Direct numerical comparisons of m_i and f_{max} to V_{cmax} and J_{max} is challenging because of the differences in resistances to CO_2 diffusion parameters in vascular plants and mosses, yet both analyses are based on a supply and demand function focusing on the differences in initial and final slopes of the photosynthesis–CO_2 relationship, thus a relative comparison between the methods is reasonable. Similar photosynthetic performance (m_i and f_{max}) in *S. caninervis* in treated and control plants after 10 years of CO_2 enrichment contrasts what is reported for most vascular plants, where acclimation to elevated CO_2 results in an average reduction of 13% in V_{cmax} and 5% in J_{max} (Long et al. 2004). Further, declines in these photosynthetic parameters are normally accompanied by a concomitant decrease in N content (Long et al. 2004, Toet et al. 2006), but in the moss examined in this study, N contents were unchanged across treatments. These data taken together suggest that desiccation-tolerant mosses, such as *S. caninervis*, do not respond to elevated CO_2 in a way similar to higher plants and do not

display photosynthetic acclimation: mosses maintain tissue N and do not increase the diffusional resistance to CO_2. Part of this may simply be due to structure (e.g. a lack of dynamic stomata) and a reliance on ambient moisture conditions to control the diffusion rate of CO_2, although efficiency of CO_2-transporting aquaporins may also play a role (Borstlap 2002, Shelden et al. 2009). The reason for maintaining levels of tissue N, however, is less clear, and we outline one possible explanation below (an allocation of N to increased thermotolerance).

Temperature had a strong effect on the photosynthetic performance of *S. caninervis* (Table 2.2A) and when subjected to 40°C, photosynthetic rates, m_i and f_{max} all declined sharply in both treatment and control plants. However, rates were depressed less in moss grown under elevated CO_2 (Fig. 2.3, Table 2.1). This suggests that the elevated CO_2 treatment in some way enhanced photosynthesis at high temperature, supporting our second hypothesis that long-term CO_2 enrichment would induce thermotolerance in *S. caninervis*.

A portion of the observed declines in performance at high temperature was likely a result of photorespiration. However, measurements were made at a constant temperature and CO_2 concentration in both CO_2-treated and control plants making it unlikely that the differences observed could be driven entirely by differential photorespiration or, if it were, it was due to CO_2-treated mosses maintaining a higher CO_2 concentration at the site of carboxylation compared to control mosses at the same ambient CO_2 concentration.

Higher f_{max} at high temperatures in mosses exposed to elevated CO_2 compared to those under ambient conditions indicates higher rates of electron transport and augmented carboxylation capacity in mosses grown under elevated CO_2. This result would be consistent with any of the following mechanisms: (1) an increase in the rate of RUBP regeneration, (2) an increase in the activity of Rubisco (Campbell and Ogren 1990, Ruuska et al. 2000) or (3) an

increased supply of elemental phosphorus (Mohr et al. 1995). Electron transport slows with increased temperatures due to destabilization of phospholipid bilayers in which the electron transport chains reside; therefore, f_{max} and the three above mechanisms associated with it would be expected to decrease as temperatures increase. Therefore, the differences in photosynthesis at high temperature observed in this study may have been driven by changes in membrane stability at high temperatures related to elevated CO_2 exposure.

PSII efficiency in S. *caninervis* (as quantified by F_v/F_m) was also 15% higher in plants grown under elevated CO_2 and is likely indicative of enhanced electron transport in plants exposed to high temperatures, despite the reduction in chlorophyll. Although F_v/F_m values for moss in this study were approximately 25% lower than typically observed in healthy vascular plants, they fall within the typical range for *Syntrichia* (*Tortula*) species (Hamerlynck et al. 2000a, 2000b) and other mosses at approximately 50% RWC (Robinson et al. 2000). When considered together, f_{max} and fluorescence analyses suggest that long-term exposure to CO_2 in *S. caninervis* resulted in increased rates of electron transport in thylakoid membranes, yet differences were only apparent at high temperature, a result strongly suggesting limitation of damage under conditions of stress.

The photosynthetic parameter, m_i, was also less diminished in moss exposed to long-term elevated CO_2 at high temperature suggesting that available CO_2 at the site of carboxylation was maintained at a higher level relative to moss under ambient conditions. We could not detect any changes in macrostructure (through measurements of shoot density and shoot length) that might indicate differences in boundary layer conductance or diffusional path length between treatments (data not shown), and samples were held at constant water content. Therefore, the influence of

the elevated CO_2 treatment on CO_2 supply is likely also reflective of stabilization of membranes that influenced the rate of CO_2 diffusion.

One suggested outcome of growing in an enriched-CO_2 atmosphere is that the photosynthetic rates are often higher at lower foliar N contents (i.e. less N is required for photosynthesis; Epron et al. 1996). In bryophytes, however, relationships between N, C and photosynthesis may not follow the same patterns as vascular plants (Rice et al. 2008). Foliar N content per unit mass of *S. caninervis* was not reduced under elevated CO_2, and if less of this N was allocated to photosynthetic function, it suggests that surplus tissue N may exist. N in excess of photosynthetic requirements could thus be allocated to other physiological functions including N-rich proteins and enzymes involved in membrane stability. Elevated CO_2 has previously been suggested to cause plants to repartition nutrients to fulfill physiological requirements (Sage 1994, Huxman et al. 1998) and change resource allocation to improve stress tolerance (Brinda et al. 2011), and the observed increased thermotolerance in *S. caninervis* may have been a result of changes in N allocation over the course of the 10-year CO_2 treatment. Under conditions where mosses have surplus substrate available for photosynthesis, N that was originally allocated to the photosynthetic apparatus could be re-allocated to protecting photosynthesis from heat damage. Elevated CO_2 may therefore influence the trade-off in N allocation between light harvesting/carboxylating mechanisms and thermotolerance of membranes in *S. caninervis*.

Moss grown in an elevated CO_2 atmosphere also exhibited a reduction in photosynthetic pigments when compared to moss grown under ambient conditions. Chlorophyll a and b levels decreased by 46%, and carotenoid (lutein and β-carotene) levels decreased by 44% on a dry weight basis (Fig. 2.1). Individually, pigment concentrations were reduced fairly uniformly across types (by 40% or more) in moss exposed to elevated CO_2. The reductions observed in this

study, especially in reference to chlorophyll, differ from the responses observed in a wide array of vascular plants from North American FACE experiments (reviewed by Long et al. 2004), where long-term growth under elevated CO_2 had no significant effect on chlorophyll content. Although little data exist on the effects of long-term exposure to elevated CO_2 on carotenoid levels, Houpis et al. (1988) noted reductions in such pigments after exposure to elevated CO_2 for 2.5 years in open-top chambers. Because carotenoids may screen UV light in addition to acting as antenna pigments, reductions in lutein and β-carotene in *S. caninervis* may reflect a reduction in ability to prevent photooxidative damage from UV in the future. On the other hand, reduced investment in antenna pigments could also eliminate excess light harvesting and could manifest in reduced damage when CO_2 assimilation drops at high temperatures.

In higher plants, short-term exposure to elevated CO_2 often results in the production of foliage with reduced chlorophyll content likely reflective of reduced demand for fixation in C_3 plants under conditions where there is increased substrate for C fixation (Houpis et al. 1988, Epron et al. 1996). However, this reduction is often not sustained (Long et al. 2004). Therefore, because of the slow growth rate of *S. caninervis* reduced pigmentation could have resulted from either a loss of pigments from existing tissues, reduced production of pigments in new tissues produced under CO_2 treatment, or a combination of both scenarios.

The ability of plants to maintain physiological function at high temperatures will be an important factor in determining future distributions of plants in arid regions (Nobel and Smith 1983, Nobel 1984), and deserts are expected to have some of the largest increases in temperature in the future. Previously, very little was known about the response of desert mosses to future atmospheric conditions. We have demonstrated that desert mosses may respond to long-term elevated CO_2 very differently than vascular plants. In particular, the lack of photosynthetic

acclimation and the potential for sustained photosynthesis under high temperature for *S. caninervis* grown under elevated CO_2 suggest the opportunity for higher rates of survival than would have been predicted under future conditions of simultaneous warming and CO_2 enrichment. Relative to present temperatures, C fixation rates will be reduced in *S. caninervis* during future high temperature scenarios, but our results suggest that CO_2-induced photosynthetic thermotolerance in this species may enable individuals to perform better than expected under these conditions. This may affect growth and biomass of *S. caninervis* within soil crust communities and because *S. caninervis* is the dominant moss in low elevation Mojave Desert biocrusts (Stark et al. 1998, Bowker et al. 2000), growth that is sustained under higher temperatures will have important ecological ramifications for C cycling in the Southwestern United States. Given that the distribution of this species extends throughout western North America (Flowers 1973) and in dry regions of Africa, Asia, the Middle East and Europe (Kramer 1980), the potential for impacts on global arid-region C cycling is likely if responses like the ones observed in this study are common across this species' broad distribution and in related biocrust moss taxa.

Acknowledgements

We wish to thank John Brinda who assisted in collection of *S. caninervis* samples from the Nevada FACE site. Additionally, gas exchange and HPLC measurements conducted at the USGS Canyonlands Research Station were greatly assisted by the support of USGS laboratory technicians, especially Sue Phillips, Matt Van Scoyoc, Krista Couch, Amanda Turner and Adam

Kind. Any use of trade names is for descriptive purposes only and does not imply endorsement by the US Government.

References

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol 165: 351–372

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant Cell Environ 30: 258–270

Bazzaz FA (1996) Plants in changing environments: linking physiological, population, and community ecology. Cambridge University Press, Cambridge

Belnap J (2003) The world at your feet: desert biological soil crusts. Front Ecol Environ 1: 181–189

Belnap J, Gillette DA (1998) Vulnerability of desert biological soil crusts to wind erosion: the influences of crust development, soil texture, and disturbance. J Arid Environ 36: 133–142

Belnap J, Phillips SL, Miller ME (2004) Response of desert biological soil crusts to alterations in precipitation frequency. Oecologia 141: 306–316

Berendse F, Van Breemen N, Rydin H, Buttler A, Heijmans M, Hoosbeek MR, Lee JA, Mitchell E, Saarinen T, Vasander H, Wallen B (2001) Raised atmospheric CO_2 levels and increased N deposition cause shifts in plant species composition and production in *Sphagnum* bogs. Glob Change Biol 7: 591–598

Berry J, Bjorkman O (1980) Photosynthetic response and adaptation to temperature in higher

plants. Annu Rev Plant Physiol 31: 491–543

Borstlap AC (2002) Early diversification of plant aquaporins. Trends Plant Sci 7: 529–530

Bowker MA, Stark LR, McLetchie DN, Mishler BD (2000) Sex expression, skewed sex ratios, and microhabitat distribution in the dioecious desert moss *Syntrichia caninervis* (Pottiaceae). Am J Bot 87: 517–526

Brinda JC, Fernando C, Stark LR (2011) Ecology of bryophytes in Mojave Desert biological soil crusts: effects of elevated CO_2 on sex expression, stress tolerance, and productivity in the moss *Syntrichia caninervis* Mitt. In: Tuba Z, Slack NG, Stark LR (eds) Bryophyte ecology and climate change. Cambridge University Press

Campbell WJ, Ogren WL (1990) Electron transport through photosystem I stimulates light activation of ribulose bisphosphate carboxylase/oxygenase (Rubisco) by rubisco activase. Plant Physiol 94: 479–484

Campioli M, Samson R, Michelsen A, Jonasson S, Baxter R, Lemeur R (2009) Nonvascular contribution to ecosystem NPP in a subarctic heath during early and late growing season. Plant Ecol 202: 41–53

Cousins AB, Adam NR, Wall GW, Kimball B, Pinter PJ, Leavitt SW, LaMorte RL, Matthias AD, Ottman MJ, Thompson TL, Webber AN (2001) Reduced photorespiration and increased energy-use efficiency in young CO_2-enriched *Sorghum* leaves. New Phytol 150: 275 – 284

Downton WJS, Berry JA, Seemann JR (1984) Tolerance of photosynthesis to high temperature in desert plants. Plant Physiol 74: 786–790

Evans RD, Ehleringer JR (1993) A break in the nitrogen cycle in arid lands? Evidence from δ15N of soils. Oecologia 94: 1432–1939

Evans RD, Lange OL (2003) Biological soil crusts and ecosystem nitrogen and carbon dynamics.

In: Belnap J, Lange OL (eds) Biological soil crusts: structure, function and management, Vol. 150. Springer, Berlin, pp 263 – 279

Epron D, Liozon R, Mousseau M (1996) Effects of elevated CO_2 concentration on leaf characteristics and photosynthetic capacity of beech (*Fagus sylvatica*) during the growing season. Tree Physiol 16: 425–432

Faria T, Wilkins D, Besford RT, Vaz M, Pereira JS, Chaves MM (1996) Growth at elevated CO_2 leads to down-regulation of photosynthesis and altered response to high temperature in Quercus suber L. seedlings. J Exp Bot 47: 1755–1761

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 149: 78–90

Flowers S (1973) Mosses of Utah new to science. Bryologist 76: 286–292

Garcia-Pichel F, Castenholz RW (1991) Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. J Phycol 27: 395–409

Garcia-Pichel F, Sherry ND, Castenholz RW (1992) Evidence for an ultraviolet sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Chiorogloeopsis* sp. Photochem Photobiol 56: 17–23 Hamerlynck EP, Huxman TE, Loik ME, Smith SD (2000a) Effects of extreme high temperature, drought, and elevated CO_2 in photosynthesis of the Mojave Desert evergreen shrub, Larrea tridentata. Plant Ecol 148: 183 – 193

Hamerlynck EP, Tuba Z, Csintalan Z, Nagy Z, Henebry G, Goodin D (2000b) Diurnal variation in photochemical and surface reflectance of the desiccation-tolerant moss, *Tortula ruralis*. Plant Ecol 151: 55–63

Heijmans MMPD, Berendse F, Arp WJ, Masselink AK, Klees H, deVisser W, van Breemen N (2001) Effects of elevated carbon dioxide and increased nitrogen deposition on bog

vegetation in the Netherlands. J Ecol 89: 268–279

Houpis JLJ, Surano KA, Cowles S, Shinn JH (1988) Chlorophyll and carotenoid concentrations in two varieties of *Pinus ponderosa* seedlings subjected to long- term elevated carbon dioxide. Tree Physiol 4: 187–193

Housman DC, Powers HH, Collins AD, Belnap J (2006) Carbon and nitrogen fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert. J Arid Environ 66: 620–634

Huxman TE, Hammerlynck EP, Loik ME, Smith SD (1998) Gas exchange and chlorophyll fluorescence responses of three south-western *Yucca* species to elevated CO_2 and high temperature. Plant Cell Environ 21: 1275–1283

Jordan DN, Zitzer SF, Hendrey GR, Lewin KF, Nagy J, Nowak RS, Smith SD, Coleman JS, Seemann JR (1999) Biotic, abiotic and performance aspects of the Nevada Desert Free-Air CO_2 enrichment (FACE) Facility. Glob Change Biol 5: 659–668

Knight CA, Ackerly DD (2002) An ecological and evolutionary analysis of photosynthetic thermotolerance using the temperature-dependent increase in fluorescence. Oecologia 130: 505–514

Knight CA, Ackerly DD (2003) Small heat shock protein responses of a closely related pair of desert and coastal *Encelia*. Int J Plant Sci 164: 53–60

Kramer W (1980) *Tortula* Hedw. sect. Rurales De Not. (Pottiaceae, Musci) in der o¨stlichen Holarktis. Bryophyt Bibl 21: 1–165

Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR (2009) Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. J Exp Bot 60: 2859–2876

Ligrone R, Duckett JG, Renzaglia KS (2000) Conducting tissues and phyletic relationships of bryophytes. Phil Trans R Soc London B Biol 355: 795–813

Lindo Z, Gonzalez A (2010) The bryosphere: an integral and influential component of the Earth's biosphere. Ecosystems 13: 612–627

Loik ME, Harte J (1996) High temperature tolerance of *Artemesia tridentata* and *Potentilla gracilis* under a climate change manipulation. Oecologia 108: 224–231

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants FACE the future. Annu Rev Plant Biol 55: 591–628

Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye AT, Gregory JM, Kitoh A, Knutti R, Murphy JM, Noda A, Raper SCB, Watterson IG, Weaver AJ, Zhao ZC (2007) Global Climate Projections. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom, New York, NY, USA

Mohr H, Schopfer P, Lawlor G, Lawlor DW (1995) Plant physiology. Springer, Heidelberg; New York

Molau U, Alatalo JM (1998) Responses of subarctic-alpine plant communities to simulated environmental change: biodiversity of bryophytes, lichens, and vascular plants. Ambio 27: 322–329

Nobel PS (1984) Extreme temperatures and thermal tolerances for seedlings of desert succulents. Oecologia 62: 310–317

Nobel PS, Smith SD (1983) High and low temperature tolerances and their relationships to

distribution of *Agaves*. Plant Cell Environ 6: 711–719

Norby RJ, Wullschleger SD, Gunderson CA, Johnson DW, Ceuelemans R (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. Plant Cell Environ 22: 683–714

Nowak RS, Ellsworth DS, Smith SD (2004) Functional responses of plants to elevated atmospheric CO_2 – do photosynthetic and productivity data from FACE experiments support early predictions? New Phytol 162: 253–280

Rice SK, Aclander L, Hanson DT (2008) Do bryophyte shoot systems function like vascular plant leaves or canopies? Functional trait relationships in *Sphagnum* mosses (Sphagnaceae). Am J Bot 95: 1366–1374

Robinson SA, Wasley J, Popp M, Lovelock CE (2000) Desiccation tolerance of three moss species from continental Antarctica. Aust J Plant Physiol 27: 379–388

Ruuska SA, Andrews TJ, Badger MR, Price GD, von Caemmerer S (2000) The role of chloroplast electron transport and metabolites in modulating rubisco activity in tobacco. Insights from transgenic plants with reduced amounts of cytochrome b/f complex or glyceraldehyde 3-phosphate dehydrogenase. Plant Physiol 122: 491 – 504

Sage RF (1994) Acclimation of photosynthesis to increasing atmospheric CO_2: the gas exchange perspective. Photosynth Res 39: 351–368

Sage RF, Sharkey TD, Seemann JR (1989) Acclimation of photosynthesis to elevated CO_2 in five C3 species. Plant Physiol 89: 590–596

Saxe H, Ellsworth DS, Heath J (2008) Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol 139: 395–436

Shaw AJ, Cox CJ, Goffinet B (2005) Global patterns of moss diversity: taxonomic and molecular

inferences. Taxon 54: 337–352

Shelden MC, Howitt SM, Kaiser BN, Tyerman SD (2009) Identification and functional characterization of aquaporins in the grapevine, *Vitis vinifera*. Funct Plant Biol 36: 1065–1078

Shreiber U (1998) Chlorophyll fluorescence: new instruments for special applications. In: Garab G (ed) Photosynthesis: mechanisms and effects, Vol. V. Kluwer Academic Publishers, Dordrecht, pp 4253–4258

Stark LR, McLetchie DN (2006) Gender-specific heat-shock tolerance of hydrated leaves in the desert moss *Syntrichia caninervis*. Physiol Plant 126: 187–195

Stark LR, Mishler BD, McLetchie DN (1998) Sex expression and growth rates in natural populations of the desert soil crustal moss *Syntrichia caninervis*. J Arid Environ 40: 401–416

Su YG, Li X, Cheng YW, Tan H, Jia R (2007) Effects of biological soil crusts on emergence of desert vascular plants in North China. Plant Ecol 191: 11–19

Taub DR, Seeman JR, Coleman JS (2000) Growth in elevated CO_2 protects photosynthesis against high temperature damage. Plant Cell Environ 23: 649–656

Toet S, Cornelissen JHC, Aerts R, van Logtestijn RSP, de Bues M, Stoevelaar R (2006) Moss responses to elevated CO_2 and variation in hydrology in a temperate lowland peatland. Plant Ecol 182: 27–40

Tuba Z, Csintalan Z, Szente K, Nagy Z, Grace J (1998) Carbon gains by desiccation-tolerant plants at elevated CO_2. Func Ecol 12: 39–44

Turetsky MR (2003) The role of bryophytes in carbon and nitrogen cycling. Bryologist 106: 395–409

Wagner D (1996) Scenarios of extreme temperature events. Clim Change 33: 385–407

Wang D, Heckathorn SA, Barua D, Joshi P, Hamilton WE, LaCroix JJ (2008) Effects of elevated CO_2 on the tolerance of photosynthesis to acute heat stress in C3, C4, and CAM species. Am J Bot 95: 165–176

Williams TG, Flannagan LB (1998) Measuring and modeling environmental influences on photosynthetic gas exchange in *Sphagnum* and *Pleurozium*. Plant Cell Environ 21: 555–564

Xu S, Liu C, Jiang P, Cai W, Wang Y (2009) The effects of drying following heat shock exposure of the desert moss *Syntrichia caninervis*. Sci Total Environ 407: 2411–2419

Zar JH (1999) Biostatistical analysis, 4th Edn. Prentice Hall, Upper Saddle River, NJ

CHAPTER 3

Precipitation-driven carbon balance controls survivorship of desert biocrust mosses[3]

[3] Originally published as: **Coe, K.K**., Belnap, J. & Sparks, J. (2012). Precipitation-driven carbon balance controls survivorship of desert biocrust mosses. *Ecology*. (in press)

Abstract

Precipitation patterns including the magnitude, timing, and seasonality of rainfall are predicted to undergo substantial alterations in arid regions in the future, and desert organisms may be more responsive to such changes compared to shifts in only mean annual rainfall. Soil biocrust communities (consisting of cyanobacteria, lichen, and mosses) are ubiquitous to desert ecosystems, play an array of ecological roles, and display a strong sensitivity to environmental changes. Crust mosses are particularly responsive to changes in precipitation, and exhibit rapid declines in biomass and mortality following the addition of small rainfall events. Further, loss of the moss component in biocrusts leads to declines in crust structure and function. In this study, we sought to understand the physiological responses of the widespread and often dominant biocrust moss *Syntrichia caninervis* to alterations in rainfall. Moss samples were collected during all four seasons and exposed to two rainfall event sizes and three desiccation period (DP) lengths. A carbon balance approach based on single precipitation events was used to define the carbon gain or loss during a particular hydration period. Rainfall event size was the strongest predictor of carbon balance and the largest carbon gains were associated with the largest precipitation events. In contrast, small precipitation events resulted in carbon deficits for *S. caninervis*. Increasing the length of the DP prior to an event resulted in reductions in carbon balance, likely because of the increased energetic cost of hydration following more intense bouts of desiccation. The season of collection (i.e., physiological status of the moss) modulated these responses, and the effects of DP and rainfall on carbon balance were different in magnitude (and often sign) for different seasons. In particular, *S. caninervis* displayed higher carbon balances in the winter than in the summer, even for events of identical size. Overall, our results suggest

annual carbon balance and survivorship in biocrust mosses is largely precipitation-driven, and because of the role mosses play in biocrusts, changes in intra-annual precipitation patterns can have implications for hydrology, soil stability, and nutrient cycling in dryland systems.

Introduction

Global climate models predict that anthropogenic warming will lead to a modest increase in mean annual rainfall within the next century (Folland et al. 2001; Stainforth et al. 2005; Held & Soden 2006; Meehl et al. 2007). However, alterations in intra-annual precipitation patterns (such as event magnitude, event frequency, and seasonality of rainfall) are predicted to change significantly for many terrestrial regions as well (Hereford et al. 2002; Meehl et al. 2007; Sun et al. 2007). On regional scales, the magnitude and direction of precipitation change is still not completely agreed upon (Meehl et al. 2007, Zhang 2007). Uncertainties are particularly apparent in predictions for arid regions (Weltzin et al. 2003), and in the case of Southwestern North America, are due to limited data and complex terrain (Hereford et al. 2002; Higgins et al. 2007).

In arid regions, water strongly limits primary productivity (Went, 1949; Noy-Meir 1973; Melillo et al. 1993; Whitford 2002; Housman et al. 2006a), and the majority of precipitation events occur as small (<5 mm) short duration events (Sala and Lauenroth 1982). Therefore, the majority of arid ecosystems exhibit a pulse-dynamic response to rainfall, where individual events offer brief pulses of resource availability for desert organisms (Huxman et al. 2004a,b) and individual rainfall events can exert a strong influence on structure and function of plant and microbial communities (Austin et al. 2004; Ogle & Reynolds, 2004; Cable et al. 2008). Some desert organisms will be relatively resilient to changes in precipitation regime because they are

adapted to extreme environments (Kleidon et al. 2000; Ward 2009). Others, already living near the physiological limits of tolerance, are likely to be particularly sensitive to changes in the features of individual events that characterize intra-annual precipitation patterns (Sala and Lauenroth 1982; Weltzin et al. 2003; Schwinning et al. 2004; Williams et al. 2006; Potts et al. 2006), regardless of the magnitude and direction of such changes.

A feature of most arid ecosystems is the presence of a biocrust living at the soil-atmosphere interface. Biocrusts are communities of cyanobacteria, lichens, and mosses (Rosentreter et al. 2007) that function collectively to increase soil stability (Belnap 2003), water holding capacity (Belnap & Lange 2001; Belnap 2003), nutrient availability (Harper & Pendelton 1993; Evans & Belnap 1999), elemental cycling (Housman et al. 2006b; Ebert et al. 2009; Grote et al. 2010), and seedling establishment of grasses and shrubs (Belnap et al. 2001; Li et al. 2005). Despite their low biomass relative to other primary producers, crust organisms have a disproportionally large influence on biogeochemistry in arid regions. Constituents of biocrusts are also extremely sensitive to environmental alteration (Belnap & Eldridge 2001; Belnap 2003; Barger et al. 2006; Ustin et al. 2009; Budel et al. 2009), and declines in diversity and biomass of crust constituent species can result in substantially reduced structural integrity of soil crusts (Belnap & Eldridge 2001) and altered soil nutrient availability (Yeager et al. 2004; Wu et al. 2009).

Biocrust mosses can play a large role in crust structure and function, and appear to be particularly sensitive to environmental change (Belnap & Eldridge 2001). For example, exposure to an increase in the frequency of small summer rainfall events has been shown to cause >90% mortality in mosses during a single growing season, and this loss of mosses led to dramatic declines in crust structure and altered biogeochemical cycling in soils (Reed et al. in press). The

sensitivity of mosses to changes in precipitation is related to the way these organisms respond to rainfall. Mosses are poikilohydric organisms for which the water content of cells is in equilibrium with the surrounding environment. Possessing neither vascular tissue nor roots, mosses are decoupled from belowground water sources, and cellular water status is determined by hydration from precipitation pulses. Physiological activity is limited to periods of hydration initiated by rainfall events, and, outside of these discrete periods of hydration, desert mosses exist in a desiccated state of suspended metabolism. Therefore, performance and growth during individual hydration events is a strong determinant of long-term persistence.

For biocrust mosses, each rainfall event induces a wet-dry cycle that is characterized by periods of net carbon (C) gain or loss (Fig. 3.1). The onset of hydration is characterized by a period of net C loss due to the respiratory costs of repair and reinstatement of metabolism. After the initiation of photosynthesis, C fixation rates surpass respiration leading to net C gains during a period of hydration. As shoots dry, a phase of C loss occurs as photosynthesis ceases and tissues are preserved for desiccation (Mishler & Oliver 2009). Therefore, in arid and semi-arid environments, each hydration event represents a balance between energy spent during rehydration and energy gained while hydrated, and ultimately results in an overall C balance that is either positive or negative. When compounded over longer time scales, C balances from individual events are directly related to long-term growth and survival.

Although intra-annual rainfall parameters such as event magnitude, frequency, and seasonality likely exert a considerable influence on the physiological ecology and survivorship of biocrust mosses, no work to date has examined how changes in such precipitation patterns influence C balance. Rainfall magnitude is typically a driver of resource availability and growth in pulse-dynamic systems and is very likely to exert a strong control on the C balance in desert

mosses. Similarly, rainfall frequency is likely to influence C balance because the timing of events is directly related to length of desiccation period between events, which is probably related to recovery cost. Further, the seasonality of rainfall likely modulates C balance because poikilohydric plants may vary in their ability to respond to hydration depending upon the season.

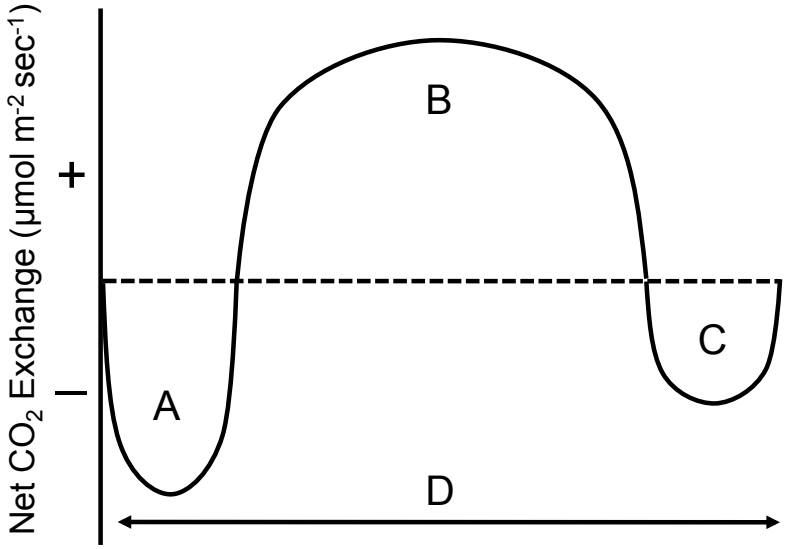


Figure 3.1: Characteristic pattern of net carbon fixation in desert moss over the course of a wet-dry cycle (of length D) initiated by a rainfall event: an initial period of net carbon loss when respiratory costs are high (A) followed by a period of net carbon gains when tissues are hydrated and photosynthesis is higher than respiration (B; length varies as a function of D) and a period of subsequent carbon loss as photosynthesis ceases and tissues prepare for desiccation again (C). Following an individual rainfall event, carbon balance is equivalent to gains from net carbon fixation (area under B) minus carbon loss from respiration (areas under A + C).

The objectives of this study were, first, to understand to what extent three intra-annual precipitation parameters (event magnitude, event frequency, and seasonality of precipitation) individually and synergistically influence C balance in the widespread and often dominant biocrust moss *Syntrichia caninervis*; and second, to measure and compare C balance values in *S. caninervis* across different rainfall event magnitudes, frequencies, and over the four seasons of

the year. Collectively, this information will allow for robust predictions of biocrust moss survivorship under future climate scenarios. We hypothesized that (1) rainfall magnitude would be the strongest predictor of C balance in *S. caninervis* because level of hydration is directly proportional to physiological activity; and (2) rainfall frequency and seasonality of rainfall would act as secondary controls on C balance. Further, we predicted that C balance values would be highest under conditions of large rainfall amounts occurring in high frequency during the winter months.

Methods

Sample collection and rainfall treatments

We used *S. caninervis* as a system in this study because: (a) it is the dominant biocrust moss on the Colorado Plateau; (b) it is widely distributed throughout drylands in the Northern hemisphere (Flowers 1973; Kramer 1980); and (c) due to morphological similarities and taxonomic relationships with other dryland mosses, physiological responses to precipitation are likely representative of other biocrust moss taxa. Mosses used in this study were collected from intact biocrusts approximately eight km South of Moab, UT on the Colorado Plateau. Here, climatic conditions include mean annual rainfall of 300 mm, mean maximum air temperatures for summer and winter of 35 and 10 °C, respectively, and an average elevation of 1550 m. Mosses were collected during spring (April 2009, May 2010) summer (July 2009, 2010) fall (October 2008, 2010) and winter (January 2008, 2009, 2010) by inserting a 2.5-cm gold-plated copper pipe cap into the moss mat and extracting a 2.5-cm diameter disc containing colonies of shoots. Due to travel logistics and available measurement time, multiple years were used for

sampling. Differences among years were tested and found to not be significant so mosses collected from multiple years in the same season were pooled for further analysis. After collection, each moss disc was rinsed of excess soil using DI water such that only soil contributing to the structural integrity of the colony remained. Discs were placed in individual 2.5-cm gold-plated copper pipe caps lined with filter paper (Whatman No. 4, Whatman/GE Healthcare corp.) and placed in labeled Petri dishes and immediately placed in a growth chamber (Heraeus Revco BK 3 m^3 No. BOD10A15, Thermo Fisher Sci., Inc., Waltham, MA). Groups of four caps were necessary for reasonable gas exchange measurements and were permanently grouped as one experimental unit for all measurements. Therefore, the total number of samples (i.e., groups of four discs) were 27, 24, 22, and 35 collected in spring, summer, fall, and winter, respectively, for a total of 108.

The primary factor influencing gas exchange in mosses is degree and length of hydration. Although desert mosses typically regain physiological and photosynthetic function within minutes of hydration (Proctor & Smirnoff 2000), field collected samples often do not exhibit statistically comparable carbon balances without some standardized hydration period prior to analysis (Coe, unpublished data; Stark, L., Belnap, J., personal communication). Therefore, in order to make comparable gas exchange measurements we standardize samples before the implementation of treatments: all samples were held in a growth chamber for five days (Daytime conditions: Photosynthetically Active Radiation (PAR) ~250 μmol m^{-2} sec^{-1}, 16.5 °C, RH ~ 45%, and a day length of ~ 9h; Nightime conditions: PAR = 0, 14.5 °C and RH artificially increased by slightly ajar Petri lids placed over the samples) and watered 5 mm twice per day.

To simulate differences in rainfall frequency in the laboratory, we used the length of dry period prior to a hydration event (referred to as desiccation period; DP) as a proxy. Samples were

exposed to either a 1-day, 5-day, or 10-day DP (n = 28, 41, and 39, respectively) prior to the rainfall event used for analysis. To ensure moss dried to the desiccated state at a natural rate, growth conditions identical to those used during pre-treatment were maintained during drying, only watering was omitted. These conditions were held constant during experimental desiccation periods.

To quantify C balance of *S. caninervis* as a function of rainfall event magnitude, samples were given either an average-size rainfall event (5 mm; n = 57) or a ¼ average size event (1.25 mm; n = 51). These event magnitudes were determined following analysis of four years of climate data from Southeastern Utah (EPA hourly climate records from CAN 407 station, 2005-2009). Events were applied directly to moss using a dropper, and samples were stored in the growth chamber at constant humidity between gas exchange measurements.

Gas exchange measurements and carbon balance analysis

To measure the per hydration event C balance of moss, we used photosynthetic gas exchange analysis to measure net C fixation to and from moss shoots over the course of an entire dry-wet-dry cycle. Approximately 10 minutes following application of the simulated rainfall event, samples of *S. caninervis* were placed in a custom nickel-plated cuvette attached to a gas-exchange system that consisted of a gas mixer (Environics Series 4000, Environics Inc., Tolland, CT), humidifier, and an infrared gas analyzer (Li-6262; Li-COR Inc. Lincoln, Nebraska) and was run with the program LabView (National Instruments Corporation, Houston, TX). Internal conditions of the cuvette were maintained at ~ 800 μmol m^{-2} s^{-1} PAR and ~ 65% relative humidity. The cuvette was temperature controlled at 17±0.5 °C using a water bath (Fisher Isotemp 105, Thermo Fisher Scientific, Inc., Waltham, MA) and a coil system built into the

cuvette. We used thermocouples to continuously monitor air and moss temperature within the cuvette over the course of the experiment (these tracked each other and never differed by >1 °C). Net C fixation was measured at hourly intervals (30 min intervals at the onset of hydration when fluxes were most dynamic) until samples had dried completely and physiological activity ceased. Samples were kept in the growth chamber between measurements, and sample weight was measured prior to and following rain events to ensure samples were dry.

Net C fixation curves were constructed using measurements over the course of dry-wet-dry cycles from simulated rainfall events. The integrated area under these curves was used to estimate total C balance for the event.

Respiration analysis

We also measured dark respiration at all net C fixation measurement intervals by extinguishing the light source, covering the cuvette with an opaque cloth and allowing samples to equilibrate for 5-10 min in the dark prior to measurement. We constructed respiration curves in the manner described above for C balance curves, and compared integrated respiration values for individual events across treatments.

Statistical Analysis

In addressing the first objective in this study (understanding how rainfall amount, DP, and season influence carbon balance and respiration in *S. caninervis*), we used linear models to determine relationships between response and predictor variables. Data for C balance and respiration were log transformed prior to these analyses to meet assumptions of normality. Although we had no *a priori* reason to suspect inter-annual variability in responses or altered

results based on small variation in seasonal sample numbers, we performed analyses to determine whether to include year (2008, 2009, 2010) as a fixed factor in our model and to examine the influence of sample size variation in seasonal responses. Inclusion of year did not significantly improve fit of C balance models and only improved Akaine Information Criterion (AIC) scores by <1 point for respiration models, therefore we treated year as a random effect in our analyses. Additionally, variation in samples sizes across seasons did not influence conclusions drawn from results, therefore we included all collected samples in our analyses.

In order to determine which combinations of our three predictor variables (rainfall amount [referred to in models as 'rain'], DP, and season) and their interactions were best able to account for the variance observed in C balance and respiration, we developed a set of candidate models using a full factorial framework, with the explicit goal of selecting the model that best represented trends in data. Our model selection criteria included AIC score analysis (a method for model selection that takes into account both likelihood and parsimony) and an r^2 selection method (based on the ability of models to explain the largest amount of variance in data). Additionally, we used partial model residuals to determine the amount of variation explained by each of the continuous predictors (rain and DP) after accounting for the effects of the other two variables.

To understand the influence of season as a categorical modulator of C balance and respiration, we analyzed these dependent variables as a function of rain and DP for each season individually. We produced equations for linear models of C balance and respiration for each season. Using differences in parameter estimates for the effects of rain, DP, and rain x DP interactions, we determined how these factors acted differently to influence C balance and respiration over the course of the year.

To address the second main objective in this study (comparing levels of carbon balance and respiration within different environmentally relevant treatment categories), we employed an analysis of variance (ANOVA). Our data, although continuous, were collected in a manner that allowed us to use a categorical ANOVA framework to examine differences in C balance and respiration values in S. caninervis across the four seasons, three desiccation periods, and two rainfall amounts. Additionally, we used Tukey's HSD tests to examine differences in mean C balance and respiration values within DP and rainfall amount categories for each season. ANOVA and linear model construction were performed using JMP v. 9 (SAS Institute, Inc. Cary, NC), and models for partial residuals were completed using the open source platform *R* (Comprehensive R Archive Network; *see* cran.r-project.org).

Results

Carbon Balance

Increasing precipitation magnitude resulted in higher C balances following events, and increasing DP length resulted in a reduction in C balance during a subsequent event (Table 3.1). On average, C balance in *S. caninervis* changed from positive to negative when the event size decreased from average to ¼ average amounts (Table 3.1, Fig. 3.2a). Average C balance values decreased significantly as DP was increased from 1 day to 10 days, and exhibited a sign change between 5 and 10 days from weakly positive values (1.65 ±2.01) to substantially negative values (-6.59±2.06; $F_{2, 105}$= 20.99; P<0.0001) (Table 3.1, Fig. 3.2a). Carbon balance also exhibited significant differences across seasons ($F_{3,104}$ = 6.04; P<0.05) with the lowest values observed in

fall and summer (-2.90±2.71 and 0.47±2.61 μmol m^{-2}, respectively) and values up to 10 times higher in magnitude occurring in winter (10.05±2.71 μmol m^{-2}; Table 3.1).

Table 3.1: Average carbon balance and respiration (μmol m^{-2} ±SE) of *Syntrichia caninervis* under different rainfall amounts, desiccation periods, and seasons (n=117 total). Different letters within treatments represent significant differences (P<0.05) in carbon balance and respiration as determined by 1-way ANOVAs and Tukey's HSD tests within treatment categories.

		Carbon Balance (μmol m^{-2})	**Respiration** (μmol m^{-2})
Rainfall Amount	5	11.41 ± 1.71 *a*	-30.06 ± 0.93 *a*
(mm)	1.25	-5.33 ± 1.80 *b*	-7.79 ± 0.98 *b*
Desiccation Period	1	14.05 ± 2.41 *a*	-16.04 ± 1.31 *a*
(days)	5	1.65 ± 2.01 *b*	-16.29 ± 1.09 *a*
	10	-6.59 ± 2.06 *c*	-24.45 ± 1.12 *b*
Season	Spring	4.54 ± 2.47 *a*	-12.66 ± 1.34 *a*
	Summer	0.47 ± 2.61 *a*	-13.66 ± 1.42 *a*
	Fall	-2.90 ± 2.71 *a*	-24.83 ± 1.48 *b*
	Winter	10.05 ± 2.17 *b*	-24.56 ± 1.18 *b*

In the case of C balance, our best model was the one considering all three variables (rain, DP, and season) and the interactions between them, which explained 76% of the variance in C balance (P <0.0001; Table 3.2). Partial residual analyses indicated that rain was the most important predictor of C balance: rain amount accounted for 79% of variance in C balance after DP and season were accounted for (Table 3.2). On the other hand, DP only explained 17% of variance in C balance after rain amount and season were taken into account (Table 3.2). Linear model equations for C balance had strong predictive power for all seasons, with r^2_{adj} values ranging from 0.59 to 0.79 (P<0.0001; Table 3.3). These models differed across seasons of the year due to differing coefficients for the predictor variables DP, rain, and the DP x rain interaction. Rainfall event magnitude significantly and positively influenced C balance in every season, DP negatively influenced C balance in fall and winter. The rain x DP interaction was significant for C balance in summer (where it was positive) and fall (where it was negative).

Table 3.2: Results from linear models using season, desiccation period (DP), rainfall amount (rain), and the interactions between these parameters, in a full-factorial design to explain variance in **(A)** C balance, and **(B)** respiration in *Syntrichia caninervis*. Models using interactions between factors also include all individual factors. Adjusted r^2 values and Akaike Information Criterion (AIC) scores show relative model fit, and partial residual (PR) adjusted r^2 values for rain and DP show variance explained by predictors after accounting for the effects of the other two variables.

A) Carbon Balance

Source	DF	Sum of Squares	*F*	*P*	r^2_{adj}	AIC	PR r^2_{adj}
Season	3	1.593	13.55	<0.0001	0.01	116.16	-
DP	1	3.237	82.66	<0.0001	0.18	93.91	0.173
Rain	1	3.131	79.93	<0.0001	0.24	85.53	0.791
Season x DP	3	3.476	29.58	<0.0001	0.41	65.21	-
Season x Rain	3	0.97	8.23	<0.0001	0.27	85.33	-
DP x Rain	1	0.082	2.10	0.1504	0.43	57.58	-
Season x DP x Rain	3	0.801	6.82	<0.0001	0.76	19.47	-

B) Respiration

Source	DF	Sum of Squares	*F*	*P*	r^2_{adj}	AIC	PR r^2_{adj}
Season	3	13.00	47.19	<0.0001	0.12	265.88	-
DP	1	4.18	45.57	<0.0001	0.04	272.95	0.519
Rain	1	49.47	538.59	<0.0001	0.64	166.53	0.823
Season x DP	3	3.28	11.91	<0.0001	0.18	262.77	-
Season x Rain	3	0.43	1.55	0.207	0.79	116.80	-
DP x Rain	1	0.003	0.03	0.865	0.68	157.35	-

Respiration

On average, respiration during periods of hydration increased as a function of rainfall amount (Table 3.1, Fig. 3.2b). Respiration decreased when the event size was reduced, and values following 1.25 mm rainfall events were ~25% of the magnitude of those following 5 mm events (P<0.05), Table 3.1, Fig 3.2b). Respiration did not differ between 1 and 5 day DP treatments but did increase by ~50% when DP was increased to 10 days ($F_{2, 105}$= 6.34; P<0.0001) Table 3.1, Fig. 3.2b). Average respiration values did not differ between spring and summer or between fall and winter, but did significantly differ *between* these groups ($F_{3, 104}$ = 10.21;

P<0.0001) with fall and winter samples showing approximately a two-fold increase in respiratory C losses (Table 3.1).

Table 3.3: Equations, r^2_{adj} values, and *P* values for linear models describing C balance and respiration (both log transformed) as a function of rain, DP, and the rain x DP interaction for spring, summer, fall, and winter. Boldface indicates terms with significant effects at the α=0.05 level.

A) Carbon Balance

Season	Linear Model Equation	r^2_{adj}	*P* value
Spring	C Balance = 3.34 – 0.01(DP) + **0.10(Rain)** – 0.004 (DP*Rain)	0.63	<0.0001
Summer	C Balance = 3.26 + 0.01(DP) + **0.06(Rain) + 0.01(DP*Rain)**	0.59	<0.0001
Fall	C Balance = 3.75 **– 0.12(DP) + 0.06(Rain) – 0.03 (DP*Rain)**	0.78	<0.0001
Winter	C Balance = 3.68 **– 0.01(DP) + 0.17(Rain)** – 0.003 (DP*Rain)	0.79	<0.0001

B) Respiration

Season	Linear Model Equation	r^2_{adj}	*P* value
Spring	Respiration = 1.01 + 0.01(DP) + **0.40(Rain)** + 0.003 (DP*Rain)	0.92	<0.0001
Summer	Respiration = 0.30 + **0.12(DP) + 0.41(Rain)** + 0.014 (DP*Rain)	0.92	<0.0001
Fall	Respiration = 1.33 + **0.10(DP) + 0.34(Rain)** + 0.004 (DP*Rain)	0.84	<0.0001
Winter	Respiration = 1.88 + 0.01(DP) + **0.34(Rain)** + 0.01 (DP*Rain)	0.77	<0.0001

In the case of respiration, our best model was also the one using all three factors (rain, DP and season) as well as the interactions between them. This model was able to account for 88% of variance in respiration, but was not significant at the a =.05 level (*P* = 0.257; Table 3.2). Rainfall amount was a strong predictor of respiration, and accounted for 64% of variance in the single factor model (*P*<0.0001). Partial residual analyses showed that rain was the most important predictor of respiration: rain amount accounted for 82% of variance in respiration after accounting for DP and season as predictors (Table 3.2). Length of DP was also a good predictor of respiration, however, and explained 52% of variance after rainfall and season were accounted for (Table 3.2).

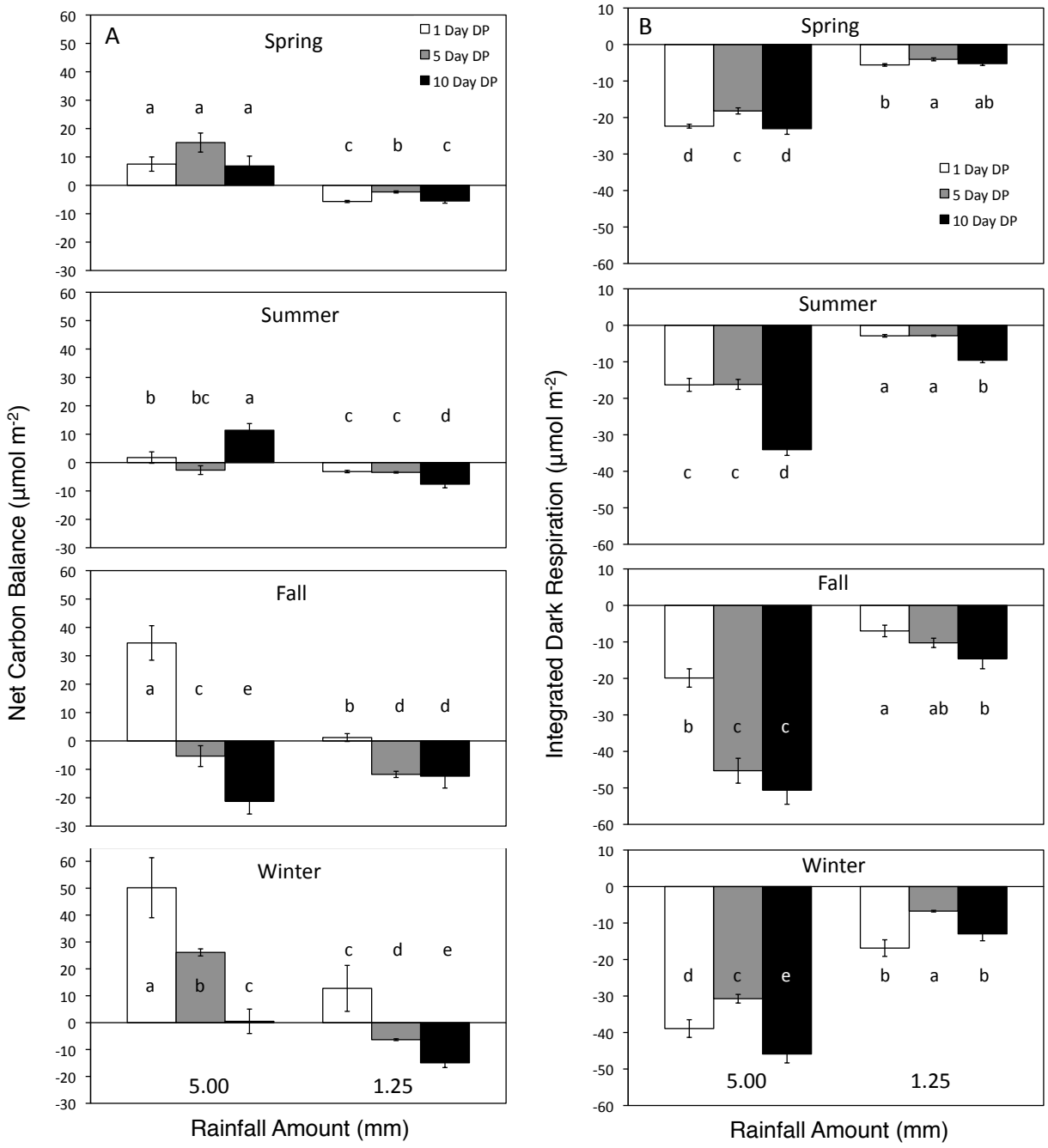


Figure 3.2: (A) Carbon balance (µmol m^{-2}) and **(B)** respiration (µmol m^{-2}) of *Syntrichia caninervis* subjected to either average (5 mm) or ¼ average (1.25 mm) rainfall amounts, following a 1-day (white bars), 5-day (gray bars), or 10-day (black bars), desiccation period (DP) and collected in Spring, Summer, Fall, and Winter. Values presented are averages (± 1 SE) of five replicates each (n=108 total) and different letters represent significant differences ($P<0.05$) in carbon balance and respiration within each season after LS means contrasts using Tukey's HSD analyses.

Seasonal linear model equations for respiration had very strong predictive power, and r^2_{adj} values ranged from 0.77 (winter) to 0.92 (spring and summer; Table 3.3). Respiration was positively influenced by rain and DP over all seasons, yet this effect was only significant for DP in fall and winter (Table 3.3).

Discussion

The intra-annual precipitation parameters rainfall magnitude, frequency, and seasonality interact to strongly influence C balance in *S. caninervis*, suggesting that alterations in one or more of these parameters can have a large effect on the physiological ecology and survival of this biocrust species under future climate scenarios.

Rainfall magnitude was positively correlated with C balance, and reducing rainfall event size from 5 to 1.25 mm resulted in both a significant reduction in C balance and, on average, a shift from C gains to C losses. This observation was driven by small events hydrating moss shoots for shorter amounts of time compared to moss shoots experiencing larger precipitation events. Small rainfall events caused moss to either stay in the active, photosynthetic phase of the wet-dry cycle (Phase B in Fig. 3.1) for a reduced period, or not enter this phase at all. In contrast, larger (5 mm) precipitation events caused mosses to enter and remain within the photosynthetic phase for longer, resulting in C gains sufficient to compensate for the initial phase of respiratory C loss (Phase A in Fig. 3.1), and leading to a positive C balance following each event. Mosses receiving 1.25 mm events, on the other hand, produced diminutive C gains that were outweighed by respiratory losses, causing C deficits.

Our results suggest that there is a minimum rainfall event size required to sustain a positive C balance in *S. caninervis,* and that this value is between 1.25 and 5 mm. Rainfall events below 2 mm may not allow mosses to fully hydrate (Stark 2005) and can lead to pigment degradation if repeated (Barker et al. 2005), supporting the notion of a minimum event size for net C gain in these organisms. Given that >70% of events in drylands are < 5 mm effective size (Sala & Lauenroth 1982; Loik et al. 2004; Reynolds et al. 2004), small carbon deficits may actually be quite common for these organisms, and desert mosses are likely reliant on the presence of a few larger events each year for long term carbon gain.

Reducing rainfall frequency resulted in a reduction in C balance of *S. caninervis* during a subsequent rainfall event. Because respiration was negatively correlated with rainfall frequency and increased significantly as DP was lengthened from 1 day to 10 days, it is probable that increasing the dry interval between rainfall events creates conditions where recovery carries a higher C cost and is more metabolically expensive. Higher respiratory losses following longer desiccation periods are likely more difficult to overcome by energetic gains from C fixation later.

We show here that when DP length increases from 5 to 10 days, C losses from respiration increase by ±50% during a subsequent rainfall event, and mosses transition from net C gains to C losses (Table 3.1). These data suggest that above a threshold desiccation length (between 5 and 10 days) the respiratory cost of hydration increases to such an extent that mosses enter C deficit from a subsequent event. Intensity of desiccation may increase with desiccation length in these environments, and recovery from desiccation bouts of higher intensity or longer duration requires more time under favorable, hydrated conditions (Hinshiri & Proctor 1971; Proctor & Smirnoff 2000; Proctor et al. 2007). Our results suggest that more energy is also required for recovery from longer periods without rain, and that unless rainfall events are large enough to

provide compensatory gains from fixation, DPs ≥10 days will consistently result in C deficit in *S. caninervis*.

We also demonstrate that responses of C balance in *S. caninervis* to rainfall amount and frequency are modulated by season. To illustrate, event magnitudes that produced positive C balances in winter often resulted in negative C balances in summer, in spite of identical measurement and pre-treatment conditions (Fig. 3.2a). Such a result may be related to fluctuations in energetic allocation to photosynthesis (and associated enzyme activity levels) over the course of the year, which is known to occur in perennial plants (Katahata et al. 2007; Koh, 2009; Kuptz et al. 2011). The Colorado Plateau receives the majority of its annual rainfall in the cool season from October to April and to a certain extent during late summer/early fall monsoon (Hereford et al. 2002). Our results suggest that the largest range and highest maximum C balance values occur during the winter and fall (Fig. 3.2a), supporting the notion of upregulated physiological response to rainfall during these times of year. Further, in contrast to most summer months when high temperatures and evaporation rates constrain the effective size of rainfall events (and water that does arrive in the form of precipitation is often likely to disappear rapidly from the system), conditions of increased humidity and reduced evaporation present in the winter result in water from precipitation events that is likely to remain available in the environment longer. Higher energetic investment in response to winter precipitation would be favorable in this environment, and while we can only speculate, our results would be consistent with *S. caninervis* exhibiting such a strategy.

The relative influence of DP on C balance and respiration also varied over the course of the year, demonstrating that changes in the timing of events in different seasons can have markedly different effects. Reducing rainfall frequency by 20%, for example, is likely to cause a

reduction in C balance and an increase in respiration that is > 10-fold greater in the fall than in the spring (Table 3.3). These relationships may result from fluctuations in energy reserves in mosses that are related to seasonal aspects of carbon assimilation. Further, in addition to constitutive protection and repair, some desert mosses possess inducible aspects of desiccation tolerance (Oliver et al. 2000) based on environmental conditions that influence rate of recovery of photosynthesis (Mayaba et al. 2001). If present in *S. caninervis*, such mechanisms operating as a function of seasonal conditions could influence the balance between C lost and C gained during precipitation events, ultimately resulting in variation in response to rainfall over the course of the year.

In general, climate change driven shifts in precipitation are uncertain for arid regions (Weltzin et al. 2003). However, we can couple the physiological responses observed in this study with modeled future precipitation patterns to form qualitative and quantitative projections under different precipitation scenarios (summarized in Table 3.4). For the Southwestern United States, most climate models predict an overall drying trend in mean annual rainfall (Hereford et al. 2002; Seager et al. 2007) caused by reductions in winter precipitation by ~10% (Meehl et al. 2007; Seager & Vecchi 2010) and an overall shift throughout the year to larger events interspersed with longer intervening drought periods (Meehl et al. 2007; Schoof et al. 2010; Scenario 1 in Table 3.4). Based on the results from this study, a low frequency rainfall scenario such as this (increasing average desiccation period by 1-3 days) would cause ~10-30% reduction in C balance of *S. caninervis*, especially if the drying trend also includes a reduction in event magnitude. Corresponding reductions in biomass would likely occur if C deficits are compounded over longer time scales. Further, biocrust mosses such as *S. caninervis* likely are reliant on C gains from precipitation events in the cooler months to compensate for losses

occurring during warmer periods, and predicted loss of winter precipitation coupled with longer droughts may result in dramatic (30-40%) annual reductions in cumulative C balance.

Table 3.4: Qualitative and quantitative predictions for annual C balance of desert mosses based on four precipitation scenarios for the next century: two broad sets of projections (Scenarios 1-2), and two individual General Circulation Models (CGMs) with strong predictive power for Southwestern North America (Scenarios 3-4). Components of each scenario were summarized based on available literature, and predictions for C balance were made based on relationships with precipitation parameters and seasonal linear models from this study.

		Annual Moss Carbon Balance Predictions		
	Estimated Components	**Independent Factor Predictions**	**Integrative Prediction**	**References**
Scenario 1	Mean annual precip 6-18%↓ Winter precip 10% ↓ 10-20% larger events 15-30% longer droughts	10-20% reduction 8-15% reduction 5-50% increase after these events, depending on season 5-25% decrease after these DPs, depending on season	Cumulative reduction (driven by mean annual and winter precipitation)	*Hereford et al. 2002* *Meehl et al. 2007* *Seager et al. 2007* *Seager & Vecchi 2010* *Schoof et al. 2010*
Scenario 2	Annual precip frequency 10-17% ↑ Intensity of monsoonal rainfall ↑	winter: 12-15% increase summer: 5-10% decrease small increase depending on event size	No change or cumulative increase; cumulative decrease if summer frequency increases proportionally more	*Higgins et al. 2007* *Schoof et al. 2010*
Scenario 3	Mean winter precip 3-10%↑ Mean spring precip 10-25%↓ Mean summer precip 30%↑	5-20% increase 5-10% decrease 0-10% increase, depending on event sizes	Cumulative reduction or no change, magnitude dependent on rainfall frequency	*GCM: Hadgem1* *Garfin et al. 2010*
Scenario 4	Mean winter precip 3-10%↑ Mean spring precip 10-25%↓ Mean summer precip 25-30%↓	5-20% increase 5-10% decrease 5-20% decrease	Cumulative reduction, magnitude dependent on rainfall frequency	*GCM: Mpi_echam5* *Garfin et al. 2010*

In contrast to the modeling studies outlined above, historical analyses suggest that a ~5% increase in rainfall frequency has occurred over the last five decades in this region (Higgins et al. 2007). Several models predict a similar trend to continue, and forecast an additional 10-15 wet days per year (representing a 10-17% increase in rainfall frequency for the Colorado Plateau) and a slight increase in intensity of monsoonal events (Higgins et al. 2007; Schoof et al. 2010; Scenario 2 in Table 3.4). This higher frequency scenario would result in favorable growth conditions in the winter months for *S. caninervis*, as even small events can produce carbon gains during this time of year, particularly if they are separated by short desiccation periods. The same scenario in the summer, however, is likely to cause repeated C deficits and cumulative losses over the course of the season. This particular mechanistic response is likely to have caused the observed >90% mortality of mosses in experiments conducted on the Colorado Plateau (Reed et al. in press).

Finally, there are two individual General Circulation Models (GCMs) with strong predictive power for precipitation patterns in Southwestern North America: Hadgem1, and Mpi_echam5 (Garfin et al. 2010; Scenarios 3 and 4, respectively, in Table 3.4). These models are somewhat limited in that they do not take into account changes in precipitation frequency, yet both forecast that in the next century mean winter precipitation will increase and mean spring precipitation will decrease. Because precipitation is a very strong predictor of C balance in *S. caninervis*, these conditions would result in increases in cumulative C balance in desert mosses during the winter, and reductions during the spring. Where these two models differ is in projections for summer rainfall: Hadgem1 forecasts small increases in summer rainfall, which is likely to produce either diminutive gains or even C losses in mosses, while Mpi_echam5 predicts reductions in rainfall, a scenario very likely to result in cumulative C losses. In spite of

contrasting predictions for summer precipitation, integrative projections from our data suggest cumulative C losses for biocrust mosses under both of these scenarios in the next century.

In conclusion, the work presented here suggests that (1) intra-annual precipitation parameters are strong predictors of C balance in desert mosses and should be considered in future efforts working to understand biocrust response to climate change; (2) surpassing minimum rainfall magnitudes and maximum desiccation periods can mean the difference between C gains and C deficits in biocrust mosses, and such thresholds may function to constrain biocrust functionality under future climate scenarios; and (3) responses to rainfall magnitude and frequency differ based on season. Despite conventional thought that increased rainfall in deserts will produce positive (even linear) ecophysiological responses in desert organisms (Kawabata et al. 2001; O'Connor et al. 2001; reviewed in Ogle & Reynolds 2004), this work illustrates that actual behavior depends on how much rain falls, how often, and during what time of year. Additionally, we demonstrate that some of the most ecologically important desert organisms can respond dramatically and nonlinearly to subtler aspects of changes in precipitation.

Acknowledgements

We wish to thank the USGS technicians Hilda Smith, Adam Kind, and Phillip Adams for invaluable assistance in sample collection and pre-treatment procedures. We also thank Ed Grote for feedback, discussion of results and continued technical support with gas exchange operations. Francoise Vermelian and Steve Ellner also offered insights and advice on statistical analyses. Any use of trade names is for descriptive purposes only and does not imply endorsement by the US Government.

References

Austin, A., Yahdjian, L., Stark, J., Belnap, J., Porporato, A., Norton, U., Ravetta, D. N., et al. (2004). Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia, 141(2), 221-235

Barger, N. N., Herrick, J. E., Zee, J., & Belnap, J. (2006). Impacts of Biological Soil Crust Disturbance and Composition on C and N Loss from Water Erosion. Biogeochemistry, 77(2), 247–263

Barker, D., Stark, L., Zimpfer, J., Mcletchie, N., & Smith, S. (2005). Evidence of drought-induced stress on biotic crust moss in the Mojave Desert. Plant, Cell & Environment, 28(7), 939–947

Belnap, J. (2003). The world at your feet: desert biological soil crusts. Frontiers in Ecology and the Environment, 1(4), 181–189

Belnap, J., & Eldridge, D. (2001). Disturbance and recovery of biological soil crusts. Biological soil crusts: structure, function, and management, 363–383. Springer

Belnap, J., & Lange, O. (2001). Structure and functioning of biological soil crusts: a synthesis. Biological soil crusts: structure, function, and management, 471–479

Belnap, J., Prasse, R, & Harper, K. T. (2001). Influence of biological soil crusts on soil environments and vascular plants. *In:* Biological Soil Crusts: Structure, Function and Management. Belnap, J & Lange, O. L. (eds). Ecological Studies, Vol. 150. Springer-Verlag Berlin Heidelberg, 2001. pp. 281-300

Büdel, B., Darienko, T., Deutschewitz, K., Dojani, S., Friedl, T., Mohr, K. I., Salisch, M., et al. (2009). Southern African Biological Soil Crusts are Ubiquitous and Highly Diverse in Drylands, Being Restricted by Rainfall Frequency. Microbial Ecology, 57(2), 229–247

Cable, J. M., Ogle, K., Williams, D. G., Weltzin, J. F., & Huxman, T. E. (2008). Soil Texture Drives Responses of Soil Respiration to Precipitation Pulses in the Sonoran Desert: Implications for Climate Change. Ecosystems, 11(6), 961–979

Elbert, W., Weber, B., Budel, B., Andreae, M.O., & Poschi, U. (2009). Microbiotic crusts on soil, rock and plants: neglected major players in the global cycles of carbon and nitrogen? Biogeosciences Discussions, 6, 6983-7015

Evans, R., & Belnap, J. (1999). Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. Ecology, 80(1), 150–160.

Flowers, S. 1973. Mosses of Utah new to science. The Bryologist 76(2): 286-292

Folland, C.K., Karl, T.R., Christy, J.R., Clarke, R.A., Gruza, G.V., Jouzel, J., Mann, M.E., Oerlemans, J., Salinger M.J., and Wang, S.-W. 2001: Observed Climate Variability and Change. In: *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change* [Houghton, J.T., Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, X. Dai, K. Maskell, and C.A. Johnson (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. pp. 99-181

Grote, E. E., Belnap, J., Housman, D. C., & Sparks, J. P. (2010). Carbon exchange in biological soil crust communities under differential temperatures and soil water contents: implications for global change. Global Change Biology, 16(10), 2763–2774

Garfin, G.M., Eischeid, J.K., Lenart, M., Cole, K.L., Ironside, K., and Cobb, N. (2010) Downscaling climate projections to model ecological change on topographically diverse landscapes of the arid southwestern United States. P. 21-44 *in* C. van Riper III, B.F. Wakeling, and T.D. Sisk, eds. The Colorado Plateau IV; shaping conservation through science and management. University of Arizona Press, Tuscon, AZ, USA

Harper, K., & Pendleton, R. (1993). Cyanobacteria and cyanolichens: can they enhance availability of essential minerals for higher plants Western North American Naturalist, 53(1), 59–72

Held, I. & Soden, B. J. (2006). Robust responses of the hydrological cycle to global warming. Journal of Climate, 19 5686-1560

Hereford, R., Webb, R. H., & Graham, S.(2002). Precipitation history of the Colorado Plateau region, 1900-2000. US Dept. of the Interior, US Geological Survey.

Higgins, R. W., Silva, V. B. S., Shi, W., & Larson, J. (2007). Relationships between Climate Variability and Fluctuations in Daily Precipitation over the United States. Journal of Climate, 20(14), 3561–3579

Hinshiri, H., & Proctor, M. (1971). The effect of desiccation on subsequent assimilation and respiration of the bryophytes *Anomodon viticulosus* and *Porella platyphylla*. New Phytologist, 527–538

Housman, D., Naumburg, E., Huxman, TE, Charlet, T., Nowak, R., & Smith, S. (2006a). Increases in desert shrub productivity under elevated carbon dioxide vary with water availability. Ecosystems, 9(3), 374–385

Housman, D., Powers, H., Collins, A., & Belnap, J. (2006b). Carbon and nitrogen fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert. Journal of Arid Environments, 66(4), 620–634

Huxman, T. E., Cable, J., Ignace, D., Eilts, J., English, N., Weltzin, J., & Williams, D. (2004a). Response of net ecosystem gas exchange to a simulated precipitation pulse in a semi-arid grassland: the role of native versus non-native grasses and soil texture. Oecologia, 141(2), 295–305

Huxman, T. E., Snyder, K., Tissue, D., Leffler, A., Ogle, K., Pockman, W., Sandquist, D., et al. (2004b). Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. Oecologia, 141(2), 1–15

Katahata, S. -I., Naramoto, M., Kakubari, Y., & Mukai, Y. (2007). Seasonal changes in photosynthesis and nitrogen allocation in leaves of different ages in evergreen understory shrub *Daphniphyllum humile*. Trees, 21(6), 619–629

Kawabata, A., Ichii K. & Yamaguchi, Y. (2001). Global monitoring of interannual changes in vegetation activities using NDVI and its relationships to temperature and precipitation, International Journal of Remote Sensing, 22(7), 1377-1382

Kleidon, A., Fraedrich, K., & Heimann, M. (2000). A green planet versus a desert world: Estimating the maximum effect of vegetation on the land surface climate. Climatic Change, 44(4), 471–493.

Koh, S. C. (2009) Novel Patterns of Seasonal Photosynthetic Acclimation, Including Interspecific Differences, in Conifers over an Altitudinal Gradient arctic and alpine research 41(3), 317

Kramer, W. 1980. *Tortula* Hedw. sect. *Rurales* De Not. (Pottiaceae, Musci) in der östlichen Holarktis. Bryophytorum Bibliotheca 21. J.Cramer, Vaduz, Liechtenstein.

Kuptz, D., Fleischmann, F., Matyssek, R., & Grams, T. E. E. (2011). Seasonal patterns of carbon allocation to respiratory pools in 60-yr-old deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees assessed via whole-tree stable carbon isotope labeling. New Phytologist, 191(1), 160–172

Li, X.-R., Jia, X.-H., Long, L.-Q., & Zerbe, S. (2005). Effects of Biological Soil Crusts on Seed Bank, Germination and Establishment of Two Annual Plant Species in the Tengger Desert (N China). Plant and Soil, 277(1-2), 375–385

Loik, M.E., Breshears, D., Lauenroth, W., & Belnap, J. (2004). A multi-scale perspective of water pulses in dryland ecosystems: climatology and ecohydrology of the western USA. Oecologia, 141(2), 269–281

Mayaba, N., Beckett, R., Csintalan, Z., & Tuba, Z. (2001). ABA increases the desiccation tolerance of photosynthesis in the afromontane understorey moss *Atrichum androgynum.* Annals of Botany, 88, 1093-1100

Meehl, G.A., T.F. Stocker, W.D. Collins, P. Friedlingstein, A.T. Gaye, J.M. Gregory, A. Kitoh, R. Knutti, J.M. Murphy, A. Noda, S.C.B. Raper, I.G. Watterson, A.J. Weaver and Z.-C. Zhao, 2007: Global Climate Projections. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Melillo, J. M., McGuire, A. D., Kicklighter, D. W., Moore, B., Vorosmarty, C. J., & Schloss, A. L. (1993). Global climate change and terrestrial net primary production. Nature, 363(6426), 234–240

Mishler, B.D., and Oliver, M.J. (2009) Putting *Physcomitrella patens* on the tree of life: the evolution and ecology of mosses. Annual Plant Reviews, 36, 1-15

Noy-Meir, I. (1973). Desert Ecosystems: Environment and Producers. Annual Review of Ecology and Systematics, 4, 25-51

O'Connor, T., Haines, L., & Snyman, H. (2001). Influence of precipitation and species composition on phytomass of a semi-arid African grassland. Journal of Ecology, 89(5), 850–860

Ogle, K., & Reynolds, J. (2004). Plant responses to precipitation in desert ecosystems: integrating functional types, pulses, thresholds, and delays. Oecologia, 141(2), 282-294

Oliver, M. J., Wood, A. J. & Mishler, B. D. (2000). The origin of vegetative desiccation tolerance in land plants. Plant Ecology, 151, 85-100

Potts, D. L., Huxman, Travis E, Enquist, B. J., Weltzin, J. F., & Williams, D. G. (2006). Resilience and resistance of ecosystem functional response to a precipitation pulse in a semi-arid grassland. Journal of Ecology, 94(1), 23–30

Proctor, M. C. F., Ligrone, R., & Duckett, J. G. (2007). Desiccation Tolerance in the Moss *Polytrichum formosum*: Physiological and Fine-structural Changes during Desiccation and Recovery. Annals of Botany, 99(1), 75–93

Proctor, M., & Smirnoff, N. (2000). Rapid recovery of photosystems on rewetting desiccation-tolerant mosses: chlorophyll fluorescence and inhibitor experiments. Journal of Experimental Botany, 51(351), 1695

Reed, S.C., Coe, K.K., Sparks, J.P., Housman, D.C., Zelikova, T.J., Belnap, J. (In press) Changes to dryland rainfall result in rapid moss mortality and altered soil fertility. Nature Climate Change

Reynolds, J., Kemp, P., Ogle, K., & Fern Ndez, R. (2004). Modifying the pulse-reserve paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. Oecologia, 141(2), 1–17

Rosentreter, R., Bowker, M., and Belnap, J. 2007. A field guide to biological soil crusts of Western U.S. drylands. U.S. Government Printing Office, Denver, CO.

Sala, OE, & Lauenroth, W. (1982). Small rainfall events: an ecological role in semiarid regions. Oecologia, 53(3), 301–304

Schoof, J. T., Pryor, S. C., & Surprenant, J. (2010). Development of daily precipitation projections for the United States based on probabilistic downscaling. Journal of Geophysical Research, 115(D13)

Schwinning, S., Sala, O., Loik, M., & Ehleringer, J. (2004). Thresholds, memory, and seasonality: understanding pulse dynamics in arid/semi-arid ecosystems. Oecologia, 141(2), 1–3

Seager, R., & Vecchi, G. A. (2010). Climate Change and Water in Southwestern North America Special Feature: Greenhouse warming and the 21st century hydroclimate of southwestern North America. Proceedings of the National Academy of Sciences, 107(50), 21277–21282

Seager, R., Ting, M., Held, I., Kushnir, Y., Lu, J., Vecchi, G., Huang, H.-P., et al. (2007). Model Projections of an Imminent Transition to a More Arid Climate in Southwestern North America. Science, 316(5828), 1181–1184

Stainforth, D. A., Aina, T., Christensen, C., Collins, M., Faull, N., Frame, D. J., Kettleborough, J. A., et al. (2005). Uncertainty in predictions of the climate response to rising levels of greenhouse gases. Nature, 433(7024), 403–406

Stark, L. (2005). Phenology of patch hydration, patch temperature and sexual reproductive output over a four-year period in the desert moss *Crossidium crassinerve*. Journal of Bryology, 27, 231–240

Sun, Y., Solomon, S., Dai, A., & Portmann, R. W. (2007). How often will it rain Journal of Climate, 20(19), 4801–4818

Ustin, S. L., Valko, P. G., Kefauver, S. C., Santos, M. J., Zimpfer, J. F., & Smith, S. D. (2009). Remote sensing of biological soil crust under simulated climate change manipulations in the Mojave Desert. Remote Sensing of Environment, 113(2), 317–328. Elsevier B.V.

Ward, D. 2009. The Biology of Deserts. Oxford University Press, Oxford, 2009

Weltzin, J. F., Loik, M. E., Schwinning, S., Williams, D. G., Fay, P. A., Haddad, B. M., Harte, J., et al. (2003). Assessing the Response of Terrestrial Ecosystems to Potential Changes in Precipitation. BioScience, 53(10), 941

Went, F. 1949. Ecology of desert plants. II.The effect of rain and temperature on germination and growth. Ecology, 30, 1-13.

Whitford, W.G. (2002) Ecology of desert systems. Academic Press, San Diego, California.

Williams, D. G., Scott, R. L., Huxman, T E, Goodrich, D. C., & Lin, G. (2006). Sensitivity of riparian ecosystems in arid and semiarid environments to moisture pulses. Hydrological Processes, 20(15), 3191–3205

Wu, N., Zhang., Y. M., & Downing, A. (2009) Comparative study of nitrogenase activity in different types of biological soil crusts in the Gurbantunggut Desert, Northwestern China. Journal of Arid Environments 73, 828-833

Yeager, C. M., Kornosky, J. L., Housman, D. C., Grote, E. E., Belnap, J., & Kuske, C. R. (2004) Diazotrophic community structure and function in two successional stages of biological soil crusts from the Colorado Plateau and Chihuahuan Desert. Applied and Environmental Microbiology. (Feb) 973-983

Zhang, X., Zwiers, F. W., Hegerl, G. C., Lambert, F. H., Gillett, N. P., Solomon, S., Stott, P. A., et al. (2007). Detection of human influence on twentieth-century precipitation trends. Nature, 448(7152), 461–46

CHAPTER 4

Predictions for dryland biocrust moss performance under future precipitation scenarios

Abstract

Drylands are likely to experience shifts in intra-annual precipitation patterns over the next century, yet little is known about how these systems will respond ecologically to these changes. In particular, changes in precipitation are likely to influence persistence of soil biocrusts (assemblages of cyanobacteria, lichen and mosses). Because of the role biocrusts play in soil fertility and stability in drylands, changes in biocrust structure and function resulting from altered performance of crust members could affect dryland ecosystem function on large spatio-temporal scales. Using the biocrust moss *Syntrichia caninervis,* we constructed a predictive model for annual cumulative carbon balance (CCB; a proxy for biomass accumulation) based on meteorological data from Southwestern North America coupled with experimentally determined physiological responses to rainfall. Using the model, we explored the influence of changes in rainfall event size, frequency, seasonality, and the number of rare large precipitation events over the next 100 years. We also ran the model using four published precipitation scenarios for drylands. Reduced rainfall event sizes, increase drought duration, and combinations of these variables decreased CCB by up to 90%. Larger rainfall event sizes and increases in the number of rare large events appeared to increase CCB by up to 150% because the carbon gains from large events sustained mosses through unfavorable environmental periods. Changes in rainfall event sizes in spring and fall had the largest effect on CCB over time, suggesting seasonal redistributions of rainfall may also influence future performance. Finally, three of four precipitation scenarios predicted by climate models resulted in 30-85% reductions in CCB in the next century, suggesting that compounded effects of different types of intra-annual precipitation alteration will cause declines in performance and survival of biocrust mosses, and emphasizing

the importance of including intra-annual precipitation in models predicting dryland response to global change.

Introduction

Increases in atmospheric greenhouse gas concentrations and subsequent warming of the Earth's surface has resulted in dramatic and ongoing changes in global precipitation patterns. Alterations in mean annual rainfall are occurring across all biomes (Trenberth et al. 2003; Stainforth et al. 2005; Alexander et al. 2006; Meehl et al. 2007; Zhang et al. 2007), and the frequency of rare large rainfall events and droughts is increasing globally (Karl & Knight 1998; Easterling et al. 2000; Alexander et al. 2006; Meehl et al. 2007). In addition, it is now understood that changes to intra-annual precipitation patterns such as individual rainfall event size, frequency at which events fall, and the seasonality of precipitation are occurring concomitantly (Hereford et al. 2002; Alexander et al. 2006; Meehl et al. 2007; Sun et al. 2007). In some ecosystems, such changes in intra-annual rainfall parameters may have larger effects on ecology and biogeochemistry than changes to mean annual rainfall alone.

Drylands (semiarid and arid systems) typically exhibit a pulse-dynamic response to precipitation where each rainfall event offers a discrete period of resource availability to organisms (Austin et al. 2004; Huxman et al. 2004; Schwinning & Sala 2004). Therefore, individual events and the features that define them are important for the physiological performance of organisms as well as the overall function of dryland ecosystems (Ogle & Reynolds, 2004; Schwinning & Sala 2004; Cable et al. 2008). While organisms living in drylands are often stress tolerators that are resilient to fluctuating environmental conditions

(Ward 2009), changes in the features of individual rainfall events (such as magnitude and timing) is one aspect of environmental alteration to which they can be extremely responsive (Sala and Lauenroth 1982; Weltzin et al. 2003; Schwinning et al. 2004; Williams et al. 2006; Potts et al. 2006).

Soil biocrusts (communities of cyanobacteria, lichens, and mosses living at the soil-atmosphere interface) are a feature of dryland ecosystems that are essential for soil stability (Belnap 2003) and biogeochemistry (Harper & Pendelton 1993; Evans & Belnap 1999; Housman et al. 2006; Ebert et al. 2009; Grote et al. 2010), yet are also among the most responsive of communities to intra-annual precipitation change (Belnap et al. 2004; Budel et al. 2009; Ustin et al. 2009). Recent evidence suggests that mosses in particular may play an important role in both the structure and function of biocrusts, yet could be the most vulnerable member of biocrusts in the face of altered precipitation regimes (Belnap & Eldridge 2001). For example, an increased frequency of small rainfall events in the summer has been shown to cause mortality in biocrust mosses after a single growing season, with dramatic declines in overall soil structure and fertility (Reed et al. in press).

Features of individual precipitation events represent a significant point of control for biocrust mosses because of the way these poikilohydric organisms respond to individual rainfall events. Biocrust mosses are desiccation tolerant plants that only hydrate and become photosynthetically active during rainfall events. Each individual rainfall event results in an intrinsic carbon balance for the moss (Green & Sancho 2011; Coe et al. 2012), and based on the magnitude, frequency, and seasonality of rainfall, events can result in either a negative or positive carbon balance (Coe et al. 2012). Survival and persistence of mosses in the biocrust community is directly related to cumulative carbon balance (CCB) from many discrete rainfall

events over long time scales. Increases in CCB in the long-term result in sustained growth for mosses, while decreases in CCB lead to declines in biomass or mortality. Developing an understanding of how different intra-annual precipitation scenarios may operate to constrain CCB in biocrust mosses will improve overall understanding of dryland response to future precipitation scenarios.

Predictions for how rainfall event magnitude, frequency, and seasonality may change over the next century in dryland systems are uncertain and variable (Weltzin et al. 2003; Meehl et al. 2007). Previous efforts to model vegetation responses to precipitation in drylands include conceptual models using the pulse-dynamic paradigm (Noy-Meir 1973, Ogle et al. 2004) and modifications to this framework that incorporate physiological responses across plant functional types (Reynolds et al. 2004). Existing models for these responses are scarce, however, and none address the direct influences of global change. Until now, predictive models for dryland system response to changes in precipitation regime for the next century (2000-2100) have not included intra-annual precipitation factors in simulations, and have not yet included biocrust organisms.

We present here a predictive model for the performance of biocrust mosses under future precipitation scenarios, with particular attention to changes in intra-annual aspects of precipitation. As an exemplar system to address modeled effects on performance, we used the biocrust moss *Syntrichia caninervis* and the drylands of Western North America (one region in which *S. caninervis* is widespread and often dominant; Rosentreter & Belnap 2001). Our objectives were to: (1) create a baseline model for biocrust moss performance under current meteorological conditions using published physiological responses to rainfall coupled with climate records from drylands in Western North America; (2) perform a series of century-long simulations to determine which intra-annual precipitation parameters have the largest influence

on future performance and survival in biocrust mosses; and (3) simulate and compare future performance in biocrust mosses using four complete predicted precipitation scenarios for Western North America.

Methods

Baseline model development

We coupled published data on the physiological responses of a biocrust moss to alterations in precipitation event amount, timing, and seasonality (Coe et al. 2012) with meteorological data from Southeastern Utah (EPA CAN 407 weather station) to form a baseline model predicting annual CCB in biocrust moss. In *S. caninervis*, rainfall event-based carbon balance is determined by the size of the rainfall event and the length of the desiccation period (DP) occurring prior to the event, and carbon balance varies over the course of the year due to physiological differences across seasons (Coe et al. 2012). Therefore, to develop the structure of the model to generate CCB we used physiology-based equations derived from relationships established by Coe et al. (2012) and calculated carbon balance as a function of (a) rainfall event size, and (b) DP, for each season of the year. As an independent test of model performance, we applied the carbon balance equations to rainfall event sizes and DP values that represented historical annual precipitation conditions (based on compiled precipitation records from Southeastern Utah). After simulating CCB in this manner for one year, values were compared to predicted CCB values based on published annual growth rates in *S. caninervis* (Stark et al. 1998). Second, we tested the ability of the model to correctly predict mortality in *S. caninervis* by simulating meteorological conditions previously hypothesized to cause physiological stress and

death in this species (Barker et al. 2005). We simulated a four-year drought period where rainfall was reduced by 30%, and tested the ability of our model to project mortality. We compared the proportion of negative moss CCB values in 100 replicates after the four-year drought to the proportion of populations exhibiting bleaching and mortality in field observations published by Barker et al. (2005).

In order to use the model construct to simulate the future biological behavior of mosses, we developed a strategy to simulate future precipitation patterns. First, we determined the natural frequencies of rainfall events and DPs of particular sizes that occur during each season of the year based on records from Southeastern Utah from 2006 to 2010. From these data, we generated probability distributions for rainfall event sizes and DPs for each season. The probability of rainfall event sizes within each season was best represented by a gamma distribution. The probability of DP lengths was best represented by a gamma distribution for spring, summer and fall, and a normal distribution for winter. To produce simulations of DPs and rainfalls over the course of a year (or for multiple years) we drew values from the rainfall event and DP distributions for each season. To change simulated rainfall event sizes or DPs from our baseline model, as in the simulations described below, we altered the parameters that define the shapes of the distributions (shape and rate for the gamma distribution, and mean and standard deviation for the normal distribution) to reflect the simulated precipitation conditions.

Simulations using individual precipitation parameters, rare large events, and projected scenarios

To determine the relative effects of intra-annual precipitation parameters on performance of biocrust mosses in the future, we manipulated four variables (rainfall event size, DP,

seasonality, and the number of rare large events) in 38 independent 100-year simulations using the model. To simulate alterations in precipitation event sizes and the length of dry period between events, we increased and decreased annual rainfall event size and DP distributions by 10%, 20%, and 30%. We altered distribution parameters for these simulations by manipulating the original meteorological data from Southeastern Utah by the desired percent reduction or increase in event size or DP, and generated novel parameters (rate or, mean and standard deviation) to be used in distributions for each season of the year. To simulate altered rainfall event sizes and frequency in our model, we drew values from these novel distributions.

To simulate an increase in either rare large precipitation events or rare long drought periods, we ran the model where 5, 10, or 15 of the simulated rainfall events or DPs in a given year were replaced with a value four times the mean annual value.

To simulate seasonal variation in rainfall event size and DP length (and determine during what time of year rainfall alterations had the greatest effect) we manipulated rainfall event size and DP to be either +30% or -30% in a single season (Spring, Summer, Fall, Winter).

To focus our studies, we ran simulations that matched as closely as possible four future precipitation scenarios predicted by prognostic modeling: Scenario 1 (+20% DP, 10 rare large rainfalls per year; *see* Hereford et al. 2002, Meehl et al. 2007, Seager et al. 2007, Seager & Vecchi 2010), Scenario 2 (-20% DP, -20% rainfall event size; *see* Higgins et al. 2007, Schoof et al. 2010), Scenario 3 (-20% rainfall event size, 10 rare long DPs per year; *see* Hereford et al. 2002, Meehl et al. 2007, Seager et al. 2007, Seager & Vecchi 2010, Schoof et al. 2010), and Scenario 4 (rainfall event sizes: +10% in winter, -20% in spring, and +30% in summer; *see* Garfin et al. 2010, Hadgem1 GCM).

All simulations were run for 100 years, using 100 independent runs (replicates) each, and CCB was compiled at 25 year, 50 year, and 100 year time points for each run. Descriptive statistics (mean, standard deviation, 5^{th}, 50^{th}, and 95^{th} percentiles) were recorded for CCB values for all time points for all simulations. Our aim in this study was to compare percent changes in CCB in each simulation with the baseline CCB for current meteorological conditions (and determine which simulated percent changes had the greatest proportional effects, compared to others). Therefore, in our analyses we focused on the percent change in CCB from baseline for each of the simulations performed.

It is likely that there are interactions between precipitation frequency, precipitation event size, and mean annual rainfall in dryland systems that can make it challenging to understand the causes of physiological responses to rainfall. For example, mean annual rainfall could be increased by (1) a decrease in average DP length annually (increasing rainfall frequency) because close temporal spacing of events could lead to an increase in the number of total events in the year, or (2) an increase in average rainfall event size. To overcome this challenge, our rainfall simulations were run with the same number of rainfall events per year, thus a decrease in average DP length over the course of a year in our model did not result in an increase in mean annual rainfall. This framework enabled us to manipulate DP independently of mean annual rainfall, and allowed us to determine the cause of changes in moss CCB on longer timescales. In terms of rainfall event size, this framework provided for environmentally relevant variation rainfall event sizes, but because the number of annual events was held constant, total annual rainfall has the possibility of being altered. This was an acceptable component of our model because, from the perspective of a moss, rainfall event size and mean annual rainfall are much more independent than for vascular plants: changes in these two variables, even if occurring in

the same direction, do not necessarily cause the same physiological effects. Overall, this method of modeling rainfall event sizes and rainfall frequency represented a compromise between running environmentally relevant simulations and the ability to determine causality of results. In spite of its caveats, this framework represents a strong step forward in modeling the effects of intra-annual precipitation in dryland systems.

Statistical analysis

Model construction, simulations, and statistical analyses were all performed using the program *R* (R Development Core Team, 2011). Percent change in CCB from baseline after 100 years was compared across the 38 simulation categories (rainfall manipulations=6, DP manipulations=6, rare large events=6, seasonal changes=16, and predicted precipitation scenarios=4). We used a one-way analysis of variance (ANOVA) to test for significant differences in percent change from baseline across simulation categories. We then used a Tukey's HSD post-hoc multiple comparison of means with an overall 95% confidence interval to analyze differences between mean percent change values in CCB across simulation categories. Finally, a multiple linear regression was run on simulation results to determine the relative influence of individual intra-annual precipitation variables on percent change in CCB. We used an α level of 0.05 in analyses unless otherwise noted.

Results

Tests of the initial model

Our initial model (using season-specific equations to calculate carbon balance) predicted annual CCB values ranging from 50-450 μmol C m^{-2} (mean = 275 μmol C m^{-2}), which fell within 95% of CCB values predicted from published annual growth rates of *S. caninervis* (Stark et al. 1998). Further, the simulation of a four-year drought period known to have caused mortality in the field (simulated rainfall was reduced by 30% over this period) resulted in negative CCB values (up to -500 μmol C m^{-2}) in 30% of the simulations. This projected mortality of 30% was similar to field observations in Nevada after a 4-year drought where 68% of populations exhibited chlorosis ranging from 28-48% (Barker et al. 2005).

Simulations

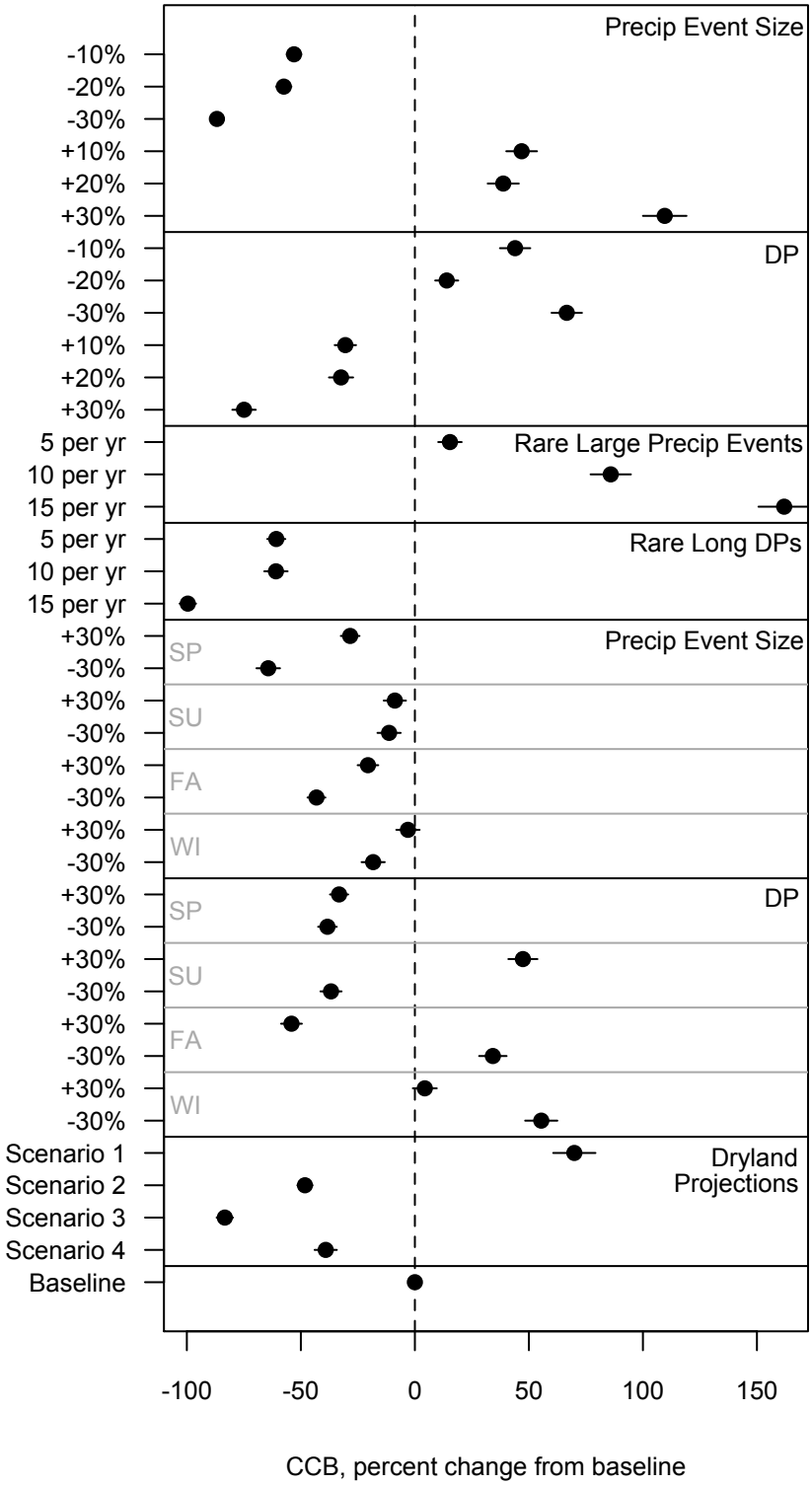
Simulated manipulations of event size, DP length, rare large events, seasonal precipitation changes, and combinatorial scenarios caused significant divergences in CCB away from the CCB predicted by baseline meteorological conditions when simulated over 100 years (P<0.001; Fig. 4.1, Table 4.1A). All differences in CCB were apparent after the first 25 years of simulation, and differences became larger in magnitude as time increased. Overall, the model was more sensitive to rainfall event size compared to DP length (Table 4.1B), with rainfall event size accounting for 32% of the variance in CCB percent change. Increasing the average rainfall event size by 10-20% resulted in a 30-40% increase in CCB and increasing the average rainfall event size by 30% resulted in a 100% increase in CCB. In contrast, reducing average rainfall event size by 10-20% resulted in a 50% reduction in CCB and reducing average rainfall event size further to 30% resulted in a 90% reduction in CCB.

Increasing DP length by 10-20% resulted in a 35% reduction in CCB and increasing DP length by 30% caused a 75% reduction in CCB. In contrast, 10-20% reductions in DP were

variable, and the increase in CCB ranged from 10% to 40%. Reducing DP length further to 30% resulted in a 65% increase in CCB (Fig. 4.1).

Manipulations of rainfall event size and DP by ±30% within individual seasons changed CCB in the 100-year simulation, and these changes were specific to season (Fig. 4.1). The model

Figure 4.1: Percent change in cumulative carbon balance (CCB) from baseline CCB (vertical dashed line) simulations of rainfall event size (±10, 20, and 30%), DP length (±10, 20, and 30%), rare large (4x average) rainfall events and rare long (4x average) DPs (+5, 10, or 15 additional per year), variation in rainfall event size and DP (±30%) across seasons (spring, SP; summer, SU; fall, FA; winter, WI), and four predicted scenarios for future precipitation patterns in Southwestern North America: Scenario 1 (+20% DP, 10 rare large rainfalls per year), Scenario 2 (-20% DP, 20% decrease in rainfall event size), Scenario 3 (-20% rainfall event size, 10 rare long DPs per year), and Scenario 4 (rainfall event sizes: +10% in winter, -20% in spring, and +30% in summer).Values presented are mean percent change in CCB (± 1 standard deviation) from baseline after 100 years of simulation.


Precip Event Size
-10%
-20%
-30%
+10%
+20%
+30%
DP
-10%
-20%
-30%
+10%
+20%
+30%
Rare Large Precip Events
5 per yr
10 per yr
15 per yr
Rare Long DPs
5 per yr
10 per yr
15 per yr
Precip Event Size
SP
+30%
-30%
SU
+30%
-30%
FA
+30%
-30%
WI
+30%
-30%
DP
SP
+30%
-30%
SU
+30%
-30%
FA
+30%
-30%
WI
+30%
-30%
Dryland Projections
Scenario 1
Scenario 2
Scenario 3
Scenario 4
Baseline
-100
-50
0
50
100
150
CCB, percent change from baseline

displayed the highest sensitivity to manipulations of spring rainfall, where CCB decreased 65% when spring rainfall event sizes were reduced 30%, and the least sensitivity to winter and summer rainfall, where CCB decreased <25% when event sizes were reduced by the same magnitude. When DP was increased by 30% in the spring and fall, the model showed a 30-50% increase in CCB. When DP was reduced by 30%, CCB was decreased by ~40% when manipulations took place in the spring and summer, and increased by 30-50% when changes occurred in the fall and winter.

Table 4.1: (A) Results from ANOVA comparing percent change in CCB from baseline across all simulation categories. (B) Results from linear regressions preformed on CCB as a function of the precipitation variables rainfall event size, DP, rare large rainfall events, and rare long DPs.

A

	DF	**Sum of Squares**	**Mean Square**	***F***	***P***
Simulation	38	12928241	340217	10736	<0.0001
Residuals	3861	122348	32		

B

Precipitation Variable	**Linear Model Coefficient Estimate**	***P***	**r^2**
Rainfall Event Size	3.034	<0.001	0.32
DP	-1.900	<0.001	0.06
Rare Large Rainfall Events	10.321	<0.001	0.39
Rare Long DPs	-5.406	<0.001	0.16

The incorporation of rare large precipitation events and rare long droughts into the model caused significant changes in CCB from baseline conditions during the 100-year simulation (P<0.001). The presence of rare large rainfall events accounted for over 30% of the variance in CCB percent change (Table 4.1B). As the number of rare large rainfall events were added to the model (5, 10, 15 events per year), CCB increased 15, 80 and 160%, respectively. As the number of rare long DPs were increased by the same increments, CCB decreased ~60% with the addition

of 5 and 10 rare long DPs, and further decreased 100% with 15 rare long DPs added to the model.

Three of the four scenarios of precipitation change based on climate projections for Western North America predicted declines in CCB in the next 100 years. Scenario 3 (-20% rainfall event size + 10 rare long DPs per year) caused the greatest reduction in CCB (-83%), and scenarios 2 and 4 showed 40-60% reductions in CCB. Scenario 1 (+20% DP + 10 rare large rainfall events) was the only scenario that produced an increase in CCB (+70%) over the next 100 years.

Discussion

From the perspective of instantaneous responses to individual rainfall events, current experimental evidence suggests that changing precipitation patterns will influence the physiology of biocrust mosses (Coe et al. 2012; Reed et al. *in press*). By extrapolating this physiological understanding over historical time using precipitation records, we created a model that successfully predicted growth and mortality in moss. The model was then used to predict future performance of mosses.

Simulations manipulating intra-annual precipitation characteristics had a large impact on moss viability. In most 100-year precipitation manipulations, changes in performance occurred in the first 25 years, indicating rapid responses to change.

Changes in rainfall event size and rainfall frequency had large effects on performance and biomass of mosses. Simulations indicate that a 30% reduction in average rainfall event size will cause a 100% decline in moss CCB in the next 100 years, and a 30% increase in average DP

will result in a 70% decline (Fig. 4.1). These two variables also largely drove the overall response to complete scenarios predicted by climate modeling for dryland systems, highlighting their importance for the physiological performance of mosses.

Rainfall event size and desiccation period are strong controlling factors of long-term CCB. However, the presence of a few rare large wet or dry events per year can override these effects and dramatically change moss CCB. Across all of the simulations, changing these precipitation extremes induced the largest (>100%) changes in CCB after 100 years. In terms of rare large (4x average) rainfall events, this appears to be due to large rainfall events driving sizeable carbon gains in desert mosses (Coe et al. 2012), thus increases in function to sustain mosses through unfavorable time periods. For example, in simulations one or two rare large rainfall events each summer enables persistence overcoming compounded carbon deficits incurred during multiple small precipitation events. On the other hand, rare long (4x average) DPs place large constraints on CCB on both short and long time scales. Longer periods of drought can lead to carbon starvation through the cost of recovery from intense periods of desiccation (Proctor 2001; Coe et al. 2012). If droughts of long duration become more common in the future, they are likely to drive declines in CCB even if rainfall event sizes do not change.

A strong emergent property of the model is that the magnitude of change in moss performance in the next century will be strongly related to the season in which precipitation patterns change. Manipulating rainfall event size in the spring or the fall had a large effect on CCB (>40 % change), but changes to rainfall event size in summer or winter had very little effect. Based on the physiological responses reported by Coe et al. (2012), this appears to be most related to: a) the seasonal physiological state of moss that influences the magnitude of carbon gains and losses from individual precipitation events; or b) the importance of the carbon

gain during rainfall events in spring and fall to compensate for losses during the summer and, to a lesser extent, the winter. The time of year that rain falls (or does not fall) therefore can have a large influence on current and future performance and biomass of biocrust mosses.

While changes to rainfall event size were more important in certain times of year than others, changes to rainfall frequency were equally important in all seasons. An increase in rainfall frequency in fall, winter, or spring led to 30-50% increases in CCB over the next 100 years, but increases during the summer resulted in 35% declines in CCB, and our data suggest that a critical rainfall frequency threshold exists for moss viability in the summer of approximately two <3 mm events per week. During the summer, experimental evidence shows that most precipitation events result in net carbon losses (Coe et al. 2012) and densely spaced events where there are more instances of loss can rapidly lead to mortality (Reed et al. in press). If future rainfall frequencies increase beyond this threshold, there are likely to be dramatic declines in performance and biomass of biocrust mosses.

Scenarios suggested by prognostic modeling for precipitation changes in North American drylands resulted in substantial changes in performance of biocrust mosses within the next century, and three out of four scenarios caused declines in viability. Scenario 1 (+20% DP, 10 rare large rainfalls per year) was the only scenario that produced a positive change in CCB. In this scenario, drought conditions imposed by the increase in DP that would ordinarily cause reductions in CCB were offset by the presence of additional rare large rainfall events per year. This provides further evidence for the importance of extremes in precipitation for long-term performance of biocrust organisms in dryland systems. An increase in number of large rainfall events, even if relatively rare, can change the trajectory of long-term performance in this species, in spite of water-limiting conditions that may co-occur.

Scenario 2 (-20% DP, -20% rainfall event size) resulted in a 48% reduction in moss CCB. In simulations altering only DP, decreased DP resulted in increased CCB likely due to reduced recovery costs from short dry periods, but in this scenario reductions in rainfall event sizes appear to have counteracted these benefits. Even if dry intervals between events are reduced, reductions in the size of rainfall events can lead to large reductions in long-term biomass accumulation. It is very likely that the results of Scenario 2 were strongly influenced by summer precipitation when most rainfall events cause carbon loss, and reductions in DP cause a reduction in CCB by compounding these deficits (see above). The results of the simulations reported here are similar to the experimental results reported by Reed et al. (in press) where the addition of small rainfall events during the summer led to moss mortality.

Scenario 3 (-20% rainfall event size, 10 rare long DPs per year) produced the largest percent decline (83%) in CCB in mosses for the next century. Reduced rainfall event size and increased frequency of extreme droughts exerted considerable influence on CCB as independent simulations. This scenario combined these variables to produce conditions that greatly limit water availability, resulting in compounded effects and dramatic declines. Collectively, the manipulation of these two parameters likely caused large reductions in CCB because (a) carbon reserves used for recovery from prolonged droughts must be replenished during rainfall events; and (b) rainfall events of insufficient size for positive carbon balance prevent this from occurring.

Scenario 4 (rainfall event sizes: +10% in winter, -20% in spring, and +30% in summer) resulted in a 40% reduction in CCB, and illustrates the importance of seasonal redistributions of precipitation on the performance of biocrust mosses. Annually, rainfall events increased in size in this scenario, but seasonally they were not increased in the time of year when biocrust mosses

are most responsive to rainfall. Therefore, increases in rainfall event size in winter and summer were insufficient to compensate for reductions in spring rainfall event sizes with respect to long-term performance. This scenario shows that alterations to the seasonality of precipitation can result in changes, and in this case, declines in performance, even if there is an overall increase in mean annual rainfall.

Overall, the model predicts that the most detrimental precipitation scenarios for biocrust moss functioning in the next century include those that reduce rainfall event size, increase drought duration, or include the combined effects of these variables (e.g., Scenario 2). Under conditions where water becomes more limiting, carbon uptake is constrained annually, leading to compounded reductions in CCB on longer time scales. In contrast, global change scenarios that drive increases in rainfall event size in dryland systems will result in higher performance and survival of biocrust moss. These scenarios could include increases in the average size of individual events, the effects of which will be most important in the spring and fall, or the presence of rare large events throughout the year. We also show that regardless of the direction of the change, responses to alterations in intra-annual rainfall can occur rapidly (<25 years) and continue on the same trajectory for a century or more.

Although predictions for changes in precipitation for drylands in North America and elsewhere globally currently come with varying degrees of uncertainty, most point to scenarios that result in reduced water availability for organisms, either through augmented drought conditions, reductions in rainfall event sizes, or seasonal redistribution of rainfall. Based on our data, such conditions are likely to result in reductions in biomass of biocrust mosses and lead to corresponding reductions in crust function. Globally, in areas with mature biocrusts that contain mosses, such changes are likely to have strong consequences for dryland ecology. In addition to

producing quantitative projections for biocrust moss functioning in a future climate, we demonstrate here that prognostic modeling of dryland biocrust function will be unable to adequately predict biocrust responses to changing precipitation regimes without an explicit consideration of the details of intra-annual precipitation patterns.

Acknowledgements

We would like to acknowledge Steve Ellner for assistance in the initial stages of development of our carbon balance model, and helpful insights throughout the simulation phase of this work. Any use of trade names is for descriptive purposes only and does not imply endorsement by the US Government.

References

Alexander, L., Zhang, X., Peterson, T., Caesar, J. Gleason, B, Klein Tank, A., Haylock, M., Collins, D., Trewin, B., Rahimzadeh, A., Tagipour, A., Kumar, K., Revadekar, J., Griffiths, G., Vincent, L., Stephenson, D., Burn, J., Aguilar, E., Brunet, M., Taylor, M., New, M., Zhai, P., Rusticucci, M., & Vasquez-Aguirre, J. (2006). Global observed changes in daily climate extremes of temperature and precipitation. Journal of Geophysical Research, 111, D05109 (22 pp)

Austin, A., Yahdjian, L., Stark, J., Belnap, J., Porporato, A., Norton, U., Ravetta, D. N., et al. (2004). Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia, 141(2), 221-235.

Barker, D., Stark, L., Zimpfer, J., Mcletchie, N., & Smith, S. (2005). Evidence of drought-induced stress on biotic crust moss in the Mojave Desert. Plant, Cell & Environment, 28(7), 939–947.

Belnap, J. (2003). The world at your feet: desert biological soil crusts. Frontiers in Ecology and the Environment, 1(4), 181–189.

Belnap, J., & Eldridge, D. (2001). Disturbance and recovery of biological soil crusts. Biological soil crusts: structure, function, and management. pp. 363–383 *in* J. Belnap and O. L. Lange, editors. Biological soil crusts: structure, function, and management. Ecological Studies 150. Springer-Verlag, Berlin, Germany.

Belnap, J., Phillips, S., & Miller, M. (2004). Response of desert biological soil crusts to alterations in precipitation frequency. Oecologia, 141(2), 306–316.

Büdel, B., Darienko, T., Deutschewitz, K., Dojani, S., Friedl, T., Mohr, K. I., Salisch, M., et al. (2008). Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency. Microbial Ecology, 57(2), 229–247.

Cable, J. M., Ogle, K., Williams, D. G., Weltzin, J. F., & Huxman, T. E. (2008). Soil Texture Drives Responses of Soil Respiration to Precipitation Pulses in the Sonoran Desert: Implications for Climate Change. Ecosystems, 11(6), 961–979.

Coe, K.K., Belnap, J. & Sparks, J.P. (2012) Precipitation-driven carbon balance controls survivorship of desert biocrust mosses. Ecology 93 (*in press*)

Easterling, D. R. (2000). Climate Extremes: Observations, Modeling, and Impacts. Science, 289(5487), 2068–2074.

Elbert, W., Weber, B., Budel, B., Andreae, M., & Poschi, U. (2009). Microbiotic crusts on soil, rock and plants: neglected major players in the global cycles of carbon and nitrogen? Biogeosciences Discussions, 6, 6983-7015

Evans, R., & Belnap, J. (1999). Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. Ecology, 80(1), 150–160.

Garfin G. M., Eischeid, J. K., Lenart, M., Cole, K. L., Ironside, K., & Cobb, N. (2010) Downscaling climate projections to model ecological change on topographically diverse landscapes of the arid southwestern United States. pp. 21– 44 *in* C. van Riper III, B. F. Wakeling, and T. D. Sisk, editors. The Colorado Plateau IV; shaping conservation through science and management. University of Arizona Press, Tuscon, Arizona, USA.

Green, T., Sancho, L., & Pintado, A. (2011). Ecophysiology of Desiccation/Rehydration Cycles in Mosses and Lichens. pp. 89-120 *in* U. Luttge et al. (eds.) Plant Desiccation Tolerance, Ecological Studies 215, Springer-Verlag Berlin Heidelberg

Grote, E. E., Belnap, J., Housman, D. C., & Sparks, J. P. (2010). Carbon exchange in biological soil crust communities under differential temperatures and soil water contents: implications for global change. Global Change Biology, 16(10), 2763–2774.

Harper, K., & Pendleton, R. (1993). Cyanobacteria and cyanolichens: can they enhance availability of essential minerals for higher plants? Western North American Naturalist, 53(1), 59–72.

Hereford, R., Webb, R. H., & Graham, S. (2002). Precipitation history of the Colorado Plateau region, 1900-2000. U.S. Geological Survey Fact Sheet 119-02. U.S. Department of the Interior, U.S. Geological Survey.

Higgins, R. W., Silva, V. B. S., Shi, W., & Larson, J. (2007). Relationships between climate variability and fluctuations in daily precipitation over the United States. Journal of Climate, 20(14), 3561–3579.

Housman, D., Powers, H., Collins, A., & Belnap, J. (2006). Carbon and nitrogen fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert. Journal of Arid Environments, 66(4), 620–634.

Huxman, T., Snyder, K., Tissue, D., Leffler, A., Ogle, K., Pockman, W., Sandquist, D., et al. (2004). Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. Oecologia, 141(2), 1–15.

Karl, T. R., & Knight, R. W. (1998). Secular trends of precipitation amount, frequency, and intensity in the United States. Bulletin of the American Meteorological Society, 79(2), 231–241.

Meehl, G., et al. (2007). Global climate projections. Pages 747–843 in S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Climate change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Noy-Meir, I. (1973). JSTOR: Annual Review of Ecology and Systematics, 4, 25-51.

Ogle, K., & Reynolds, J. (2004). Plant responses to precipitation in desert ecosystems: integrating functional types, pulses, thresholds, and delays. Oecologia, 141(2), 1–13.

Potts, D. L., Huxman, T. E., Enquist, B. J., Weltzin, J. F., & Williams, D. G. (2006). Resilience and resistance of ecosystem functional response to a precipitation pulse in a semi-arid grassland. Journal of Ecology, 94(1), 23–30.

Proctor, M. (2001). Patterns of desiccation tolerance and recovery in bryophytes. Plant Growth Regulation, 35(2), 147–156.

R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Reed S. C., Coe, K.K., Sparks, J. P., Housman, D. C., Zelikova, T. J. & Belnap, J. *In press*. Changes to dryland rainfall result in rapid moss mortality and altered soil fertility. Nature Climate Change.

Reynolds, J., Kemp, P., Ogle, K., & Fern Ndez, R. (2004). Modifying the pulse-reserve paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. Oecologia, 141(2), 1–17.

Rosentreter, & Belnap, J. (2001) Biological Soil Crusts of North America. In Belnap, J. & Lange, O. L. (eds) Biological soil crusts: structure, function, and management. Ecological Studies,Volume 150, Part I, 2001. Springer-Verlag Berlin Heidelberg, 2001. pp. 31-50

Sala, O.E., & Lauenroth, W. (1982). Small rainfall events: an ecological role in semiarid regions. Oecologia, 53(3), 301–304.

Schoof, J. T., Pryor, S. C., & Surprenant, J. (2010). Development of daily precipitation projections for the United States based on probabilistic downscaling. Journal of Geophysical Research, 115, D13106.

Schwinning, S., & Sala, O. (2004). Hierarchy of responses to resource pulses in arid and semi-arid ecosystems. Oecologia, 141, 211-220.

Schwinning, S., Sala, O., Loik, M., & Ehleringer, J. (2004). Thresholds, memory, and seasonality: understanding pulse dynamics in arid/semi-arid ecosystems. Oecologia, 141(2), 1–3.

Seager, R., & Vecchi, G. A. (2010). Climate change and water in southwestern North America special feature: Greenhouse warming and the 21st century hydroclimate of southwestern North America. Proceedings of the National Academy of Sciences, 107(50), 21277–21282.

Seager, R., Ting, M., Held, I., Kushnir, Y., Lu, J., Vecchi, G., Huang, H.-P., et al. (2007). Model projections of an imminent transition to a more arid climate in southwestern North America. Science, 316(5828), 1181–1184.

Stainforth, D. A., Aina, T., Christensen, C., Collins, M., Faull, N., Frame, D. J., Kettleborough, J. A., et al. (2005). Uncertainty in predictions of the climate response to rising levels of greenhouse gases. Nature, 433(7024), 403–406.

Stark, L.R., Mishler, B.D., & McLetchie, D.N. (1998). Sex expression and growth rates in natural populations of the desert soil crustal moss *Syntrichia caninervis*. Journal of Arid Environments, 40, 401-416

Sun, Y., Solomon, S., Dai, A., & Portmann, R. W. (2007). How often will it rain? Journal of Climate, 20(19), 4801–4818.

Trenberth, K. E., Dai, A., Rasmussen, R. M., & Parsons, D. B. (2003). The Changing Character of Precipitation. Bulletin of the American Meteorological Society, 84(9), 1205–1217.

Ustin, S. L., Valko, P. G., Kefauver, S. C., Santos, M. J., Zimpfer, J. F., & Smith, S. D. (2009). Remote sensing of biological soil crust under simulated climate change manipulations in the Mojave Desert. Remote Sensing of Environment, 113(2), 317–328.

Ward, D. 2009. The Biology of Deserts. Oxford University Press, Oxford, 2009

Weltzin, J. F., Loik, M. E., Schwinning, S., Williams, D. G., Fay, P. A., Haddad, B. M., Harte, J., et al. (2003). Assessing the response of terrestrial ecosystems to potential changes in precipitation. BioScience, 53(10), 941.

Williams, D. G., Scott, R. L., Huxman, T. E., Goodrich, D. C., & Lin, G. (2006). Sensitivity of riparian ecosystems in arid and semiarid environments to moisture pulses. Hydrological Processes, 20(15), 3191–3205.

Zhang, X., Zwiers, F. W., Hegerl, G. C., Lambert, F. H., Gillett, N. P., Solomon, S., Stott, P. A., et al. (2007). Detection of human influence on twentieth-century precipitation trends. Nature, 448(7152), 461–465.